



University of Zabol

# Agriculture, Environment & Society

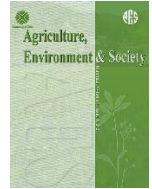


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## **Editor Preface to the First Issue**

On behalf of the Agriculture, Environment & Society (AES) editorial board and my colleagues, Dr. Daniel E. Campbell, Dr. Esmael Seyedabadi, and Dr. Mojtaba Keykhasaber, I am thrilled to announce the publication of the inaugural issue of the Journal Agriculture, Environment & Society (AES).

The AES is a new publication that seeks to establish a presence in the already crowded field of scientific journals devoted to three important systems that we must understand that will move Iran forward and improve the living conditions of people: the environment, agriculture, and society. AES is an international peer-reviewed Journal published under the Open Access scheme, which grants free access to the published papers by University of Zabol, Iran. Unlike the majority of Open Access journals, however, AES does not charge for page or publication charges. This almost unique combination of free publishing and open access would allow for the dissemination of high-quality scientific papers to the scientific community while ensuring a rigorous peer-review procedure overseen by an editorial board of twenty highly reputed scientists.

The first issue of the journal hosts ten research papers on contemporary environmental issues in Iran. The papers vary from industrial diagnostics to environmental analysis to cultural heritage studies, revealing the extraordinarily broad variety of approaches and applications that can be applied to the country's current needs in order to advance the Iranian people's well-being.

While we appreciate the authors of the papers published in this inaugural issue, we welcome researchers working in the fields of agriculture, the environment, and society to consider submitting their manuscripts to our Journal for publication. We will make every effort to maximize the visibility of their work while simultaneously increasing the effect of our Journal on the Scientific Community working in the subject.

Again, I would like to thank all colleagues collaborating in this project as well as Dr. Majid Erfanian, Dr. Ali Emanjomeh and Dr. Reza Zaboli at the Vice Chancellor for Research of the University without whose unflagging support this work would not have taken off.

**Mohammad Reza Asgharipour**

**Editor in Chief**

**Agriculture, Environment & Society (AES)**

## Aims and Scopes

*Agricultural, Environment and Society* 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.

*Agricultural, Environment and Society* 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 *Agricultural, Environment and Society*:**

- *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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## Effects of different cotton tillage methods on N<sub>2</sub>O and NH<sub>3</sub> emissions in a cotton-wheat rotation

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### ABSTRACT

Conventional tillage methods result in resource waste and the release of greenhouse gases into the environment. An experiment with a randomized complete block design and three treatments in four replications was conducted at Darab Agricultural Research Station for five years to determine the effects of different tillage methods on nitrous oxide (N<sub>2</sub>O) and ammonia (NH<sub>3</sub>) emissions in cotton-wheat rotation. Direct farming (no tillage), low tillage, and conventional wheat-cotton tillage (control) were used as treatments. Following wheat harvesting in the direct and low tillage treatments, 30% (weight) of wheat residues were dispersed on the field. Within two years, N<sub>2</sub>O and NH<sub>3</sub> emissions from the cotton-wheat field were estimated using the DNDC 9.5 model. Data from the first three years of the study was used to validate the model. The results of model validation revealed that the model performed well in simulating the soil environment as well as N<sub>2</sub>O and NH<sub>3</sub> emissions. The simulation results revealed that the highest and lowest N<sub>2</sub>O emission rates occurred in conventional and no-tillage treatments, with a significant difference. After five years of experimentation, average annual N<sub>2</sub>O emissions were 4.40, 2.80, and 2.14 kg N ha<sup>-1</sup> y<sup>-1</sup> for conventional, low, and no-tillage treatments, respectively. According to the simulation results, peak NH<sub>3</sub> emission from soil occurred on the fifth day after fertilization in all three treatments. The overall findings of this study indicated that the use of no-tillage methods is more advantageous than other cotton culture treatments in cotton-wheat rotation under similar conditions as in the current study.

### 1. Introduction

Environmental issues have received increasing attention in recent years. Different economic sectors are interested in evaluating the effects of their activities on increasing environmental awareness. Agriculture is one of the most important economic sectors that has important effects on the environment (Charles et al., 2017). Increasing mechanization, improving production methods, widespread application of fertilizers and pesticides, and improvements in animal husbandry during the 20<sup>th</sup> century have contributed to the increase in production. Today, energy consumption in the agricultural sector has increased as a result of population growth, reduced arable land, and improved welfare levels. Intensive use of chemical fertilizers, pesticides, agricultural machinery, electrical energy, and natural resources is needed to provide food for

the growing population (Fittton et al., 2017). Meanwhile, fossil resources are limited, so it is imperative to preserve these resources for future generations of human beings through proper and high-efficiency consumption. On the other hand, intensive use of land sources causes environmental problems. Agriculture is the main source of several important environmental pollutants (Franqueville et al., 2018). According to the Kyoto Treaty (1997), agricultural development with high-efficiency energy consumption can be effective in reducing greenhouse gas emissions from agricultural activities. Optimum use of resources in agriculture reduces environmental problems, prevents the degradation of natural resources, and expands sustainable agriculture (Deng et al., 2018).

Agriculture is a well-known and substantial source of greenhouse gas emissions (Bareau et al., 2017). The

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concentration of atmospheric nitrous oxide ( $N_2O$ ) has risen by 20% since 1750 AD (Ciais et al., 2013). Nitrogenous fertilizer application and agronomic operations account for 78% of  $N_2O$  emissions in the United States (UNEP, 2013). Agricultural activities account for 90% of the total anthropogenic  $NH_3$  emissions in Canada, and  $NH_3$  emissions have increased by 23% since 1990 due to increasing fertilizer use in agriculture and animal husbandry (Congreves et al., 2016).

The complexity of the relationships governing biogeochemical cycles and their importance leads to the application of predictive models to study the impacts of climate change and land use on the emission rates of greenhouse gases and changes in soil characteristics to discover the truth (Li et al., 2009). The study of the carbon and nitrogen cycles and their effects on global climate allows for the use of models to inspect the amount of greenhouse gas emissions into the atmosphere and investigate options for reducing agriculture's role in climate change. The DNDC (DeNitrification-DeComposition) model provides satisfactory results in simulations of carbon and nitrogen cycles and greenhouse gas emissions from agricultural lands. This model is written using the C++ programming language and includes two main modules. The first module can simulate crop growth and carbon and nitrogen cycles in the soil using three sub-models of soil climate, crop growth, and soil degradation (Uzoma et al., 2015). The second module can simulate the biochemical processes associated with soil environmental factors using nitrification, denitrification, and fermentation sub-models. The DNDC divides soil carbon stocks into four parts: plant residues, plant biomass, active humus, and inactive humus. In the next step, carbon stocks are divided into three sub-sections, *viz.*, highly unstable, unstable, and resistant, based on the differences in carbon to nitrogen ratios and decomposition rates (Li, 1994, 1995). This model utilizes the classical laws of physics, chemistry, and biology in conjunction with empirical equations derived from laboratory studies to determine the soil-plant biogeochemical parameters (Giltrap et al., 2010).

Inputs required for the DNDC model implementation include the study site (including geographical coordinates), daily meteorological information (minimum and maximum temperatures, precipitation, wind speed, solar radiation, and relative humidity), soil physical properties (texture, soil water content at the field capacity point and permanent wilting, apparent soil weight, soil reaction, soil hydraulic conductivity, and soil mineral nitrogen and organic carbon contents), and management operations (crop rotation, tillage operations, details of chemical and organic fertilizer applications, planting and harvesting dates, and irrigation methods). Plant growth and yield, allocation of plant biomass to leaves, stems, roots, and seeds, nitrification and denitrification, soil temperature and moisture profiles, soil carbon reservoirs and fluxes, soil nitrogen reservoirs and fluxes, nitrate leaching, and the emissions of carbon dioxide ( $CO_2$ ), methane ( $CH_4$ ), ammonia ( $NH_3$ ), nitric oxide ( $NO$ ), and nitrous oxide ( $N_2O$ ) from plant-soil systems are simulated and modeled at the end of each day by running the DNDC model. The DNDC also simulates

and provides an annual report for the system based on the annual crop yield as well as carbon, nitrogen, and water reservoirs and fluxes (Li, 2000).

Agricultural activities, such as plowing and management of crop residues, play an important role in determining the crop yield and/or greenhouse gas emissions (Pandey et al., 2012; Liu et al., 2014). Many researchers have investigated the effects of plowing and tillage operations on the emissions of greenhouse gases. Pandey et al. (2013), for instance, examined the effects of different tillage methods on wheat-rice rotation in India and reported that conventional plowing for both plants increased greenhouse gas emissions in addition to increasing wheat yield. In another study, the same authors (Pandey et al., 2012) found that tillage reduction could reduce the release of  $CH_4$ ,  $N_2O$ , and crop yield.

Soil tillage and management of residues can change the dynamics of carbon (C) and nitrogen (N), resulting in significant changes in greenhouse gas emissions and crop yields (Smith et al., 2011; Li et al., 2010). Experiments in China for 50 years showed that increasing farm residues reduced greenhouse gas emissions during the growing season (Song et al., 2019). Agriculture is expected to have a lower effect on  $N_2O$  emissions through the application of irrigation and fertilization management methods (Mielenz et al., 2016). West and Marland (2002) reported that average carbon emissions for soybean production in conventional tillage, low-tillage, and no-tillage systems were reported as 168, 146, and 137 kg ha<sup>-1</sup>, respectively (West and Marland, 2002). Organic carbon and total nitrogen were uppermost in the no-tillage system, followed by the low-tillage system, with the least amount in the conventional tillage system. This was attributed to increasing enzymatic activity in the no-tillage system due to less soil disturbance (Mohammadi et al. 2012). Researchers reported more  $N_2O$  emissions for conventional tillage compared to low or no tillage applications. However, Rochette et al. (2008) observed increased emissions of nitrogen oxides from soils compressed by tillage, particularly in loamy soils. Zhang et al. (2015) experimented with different effects of tillage and residue management on greenhouse gas emissions in China. They reported that although  $CH_4$  and  $N_2O$  emissions were not significantly different in various treatments during the wheat growth season,  $CH_4$  emission rates were significantly different between treatments in the rice growing season. Also, the interaction effect of tillage and residue management was significant on greenhouse gas emissions, but had no significant impacts on wheat and rice yields.

It is known that denitrification and  $N_2O$  production increase with rising water-filled pore space (WFPS). Maximum  $N_2O$  emission is achieved at WFPS values above 70%, and maximum denitrification occurs at soil saturation (Liang et al., 2018). Some studies have shown that  $N_2O$  emission rates in conventional tillage and no-tillage systems are controlled by the soil water content (Boeckx et al., 2011; Almaraz et al. 2009), but the positive or negative effects of tillage on  $N_2O$  emission are largely dependent on soil texture and atmospheric conditions

(Fang et al., 2015). Almaraz et al. (2009) studied soy plants in Canada and found that N<sub>2</sub>O emission decreased from a maximum of 18.1 mg m<sup>-2</sup> day<sup>-1</sup> in conventional tillage to 7.4 mg m<sup>-2</sup> day<sup>-1</sup> in a no-tillage system. They also reported that increasing soybean N<sub>2</sub> fixation in a no-tillage system might reduce CO<sub>2</sub> and N<sub>2</sub>O emissions compared to conventional plowing. Existing field studies, however, do not clearly illustrate the effects of tillage or management of residues and/or the interaction of these two methods on greenhouse gas emissions (Bayer et al., 2014).

Considering the cost of direct measurements and the high error of point measurements, a cost-effective method is necessary to determine optimal soil management practices and their effects on atmospheric carbon sequestration and greenhouse gas emissions reduction using simulation models with proven capability. This issue is important in assessing carbon output and the role of agricultural soils in the formation and emission of

greenhouse gases and the development of sustainable agriculture. Accordingly, this research aimed to evaluate the impacts of different tillage systems on N<sub>2</sub>O greenhouse gas emissions in cotton-wheat rotation using the DNDC model.

## 2. Materials and methods

### 2.1. The study area and experimental design

This study was conducted at the Hassan Abad Agricultural Research Station of Darab, which is affiliated with the Fars Agriculture and Natural Resources Research Center (57° 54' E and 29° 28' N, with 1107 m above sea level) and is located 230 km south-east of Shiraz, in a warm-dry climate with long-term rainfall (285 mm). The soil specification of the study site is presented in Table 1. The soil texture was loamy, and the studied land had been fallow for three years before the experiment onset.

**Table 1. Soil physicochemical characteristics in the study site**

Depth (cm)	EC (dS m <sup>-1</sup> )	pH	O.C. (%)	N (mg kg <sup>-1</sup> )	Ab. P	Ab. K	Silt	Clay	Sand	Soil texture
0-15	1.7	7.8	0.58	22	5.9	149	47.5	17.1	35.4	Loam
15-30	0.76	7.8	0.52	24	4.7	97	46.1	19.1	34.8	Loam

This experiment aimed to determine the effects of different tillage methods on greenhouse gas emissions in cotton cultivation in a completely randomized block design with three treatments in four replications during five crop years (2012–2017). The research treatments included direct (no-tillage) wheat-cotton cultivation, low-tillage wheat-cotton, and conventional tillage of wheat-cotton as a control. The dimension of each experimental plot was 180 m<sup>2</sup> (30 m long and 6 m wide). Each wheat plot consisted of 40 planting rows cultivated with inter-line and on-line spacing of 15 cm and 2 cm, respectively, in the last week of November in all 5 years. Wheat was harvested with a special experimental combine and 30% (weight) of wheat residues were dispersed in the field at conservational tillage treatments (low and no tillage). In cotton cultivation, each plot included eight planting rows with an inter-line and on-line spacing of 70 cm and 20 cm, respectively, in the last week of April in all five years.

In direct farming (no-tillage), no tillage operations were done before planting, and the plants were cultivated with one movement of a direct planter (Semeato, model SHM 11/13, Brazil) in the field. A compound tiller (Mark Puttinger, Austria) was used in the low-tillage method. Wheat and cotton were cultivated by a grain drill and a row-planter, respectively. In the conventional method, soil tillage was carried out by moldboard and disk plows, and wheat and cotton were cultured with a line-planter and a row-planter, respectively.

All stages of cotton production were performed according to the instructions of the Iranian Cotton Institute. The farm was irrigated using a siphon in such a way that the height of water behind the siphons was constant and at a similar level for all siphons. Similar irrigation durations were considered for all treatments. The amounts of fertilizers were determined based on the soil test results in all 5 years. Levels of fertilizer use were similar in wheat

and cotton fields, and all superphosphate fertilizer (30 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>), potassium nitrate (44 kg ha<sup>-1</sup> of K<sub>2</sub>O), and one-third of urea fertilizer (60 kg ha<sup>-1</sup> of pure N) were applied to the plots by the planter at the cultivation time. The rest of the urea fertilizer was spread as top-dress in the field in two steps (after thinning and after flowering). For weed control on cotton farms, Treflan herbicide (4 L ha<sup>-1</sup>) was used as pre-culture together with the first irrigation. In addition, manual weeding was carried out at two stages, one in the four-leaf stage along with thinning (40 days after planting) and the other in the pre-flowering stage.

In the cotton field, after full opening of the bolls at the end of the growth season, cotton seed per plot was harvested in separate sacks and weighed with a digital scale to determine the cotton yield. At harvest time, the cotton biomass was calculated by cutting the plants from the soil surface and drying them in an oven at 70 °C for 48 h.

Average soil water content during the experimental period was measured using a TFA max-min thermometer (IP67, Germany). To calculate WFPS (%) (Paul, 2007), soil water content was measured as volumetric water content (VWC) in m<sup>3</sup> m<sup>-3</sup>.

$$WFPS = \frac{VWC}{1 - BD/PD} \times 100\% \quad \text{Eq.1}$$

Soil bulk density (BD) was 0.83 g cm<sup>-3</sup> at a depth of 5 cm, and a particle density (PD) of 2.65 g cm<sup>-3</sup> was considered in this study. Meteorological data was obtained from the weather station in Hasanabad, Darab.

### 2.2. Measurement of N<sub>2</sub>O and NH<sub>3</sub> emissions

The N<sub>2</sub>O and NH<sub>3</sub> emission rates were estimated by placing a cap on the soil at weekly intervals. In this method, each cap was inserted into the soil (2 cm) and N<sub>2</sub>O and NH<sub>3</sub> fluxes were sampled in a Plexiglas chamber after 30 minutes. The samples were then transferred to the

laboratory to determine the N<sub>2</sub>O content through gas chromatography (GC) (Model 14a-Shimadzu, Japan). Ammonia emission rates were also measured using the Bentech ammonia assay (GM8806, China). The emission of gases was simulated by the DNDC 9.5 model.

### 2.3. Statistical evaluation of simulation results

The predictive power of the model was evaluated using the normalized root mean square errors (RMSEn) and error regression coefficients of actual values against predicted values. RMSEn was calculated using Equation 2 (Rinaldi et al., 2003).

$$RMSE_n = 100 \left( \sum_{i=1}^n (P_i - O_i)^2 / n \right)^{0.5} / O_{mean} \quad \text{Eq. 2}$$

In this equation, P<sub>i</sub> and O<sub>i</sub> are, respectively, the predicted and actual values, n is the real number of actual measurements of the plant components, and O<sub>mean</sub> is the actual mean value. RMSEn is expressed as a percentage difference between the predicted and actual values. By definition, RMSE values of < 10%, 10-20%, 20- 30%, and >30% indicate excellent, good, moderate, and weak estimations of the model's predictive power (Rinaldy et al., 2001). Excel software was used for fitting the equations and statistical calculations.

### 2.4. Model calibration

To design a model, it is necessary to have a rather complete understanding of the processes, mathematical equations, and algorithms describing the processes, since most biogeochemical models need to adapt and adjust the parameters controlling processes (e.g., N<sub>2</sub>O and CO<sub>2</sub> generation, leaching, soil moisture storage, etc.). Model regulation or calibration involves the parameters for model coordination and compatibility in producing such data as gas emissions or the original system specimen. In other words, the calibration aims to minimize the difference between the predicted and observed output, and this may be done by accurate measurement of parameters or with optimization methods. There is usually a special relationship between the general model form and the physical system studied through the model parameters, which determines the accuracy of parametric values for a certain proportion between the model output and the measured output.

In this study, the model was calibrated by test and error (manual method). In this method, the included measurable parameters were soil temperature and moisture, as well as cotton biomass and yield. Unknown parameters were estimated as preliminary estimates. Then, the model was run and its output was compared with that observed in the original sample. In this study, data from the first three years of research was used to calibrate the model.

## 3. Results and discussion

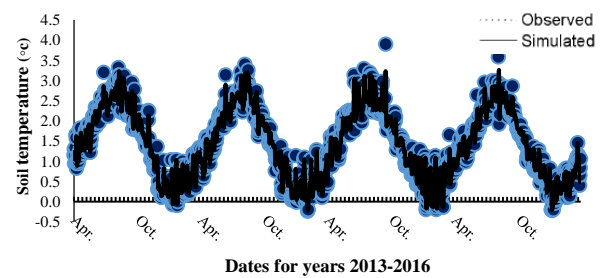
### 3.1. Soil temperature and moisture

Figure 1 shows the simulated and observed values of average daily soil temperatures at a depth of 5 cm in the three treatments from April 2013 to March 2016. Temporal

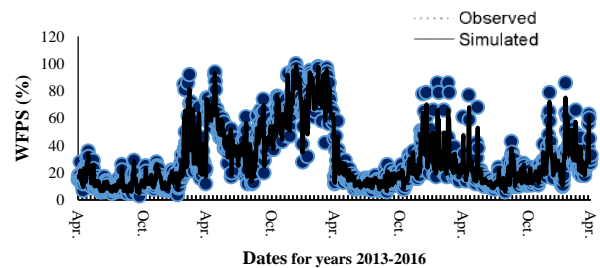
patterns and simulated values were consistent with field measurements. A linear correlation coefficient (R<sup>2</sup>) of 0.97 was obtained for the simulated values against the observed average daily temperatures with a slope of 1.0 (p<0.01) and an RMSE value of 10.13% (Table 2).

**Table 2. Statistics of simulated DNDC variables in comparison to observed values**

Variables	Linear regression				RMSE (%)	n
	Intercept	Slope	R <sup>2</sup>	p		
Cotton biomass (Mg ha <sup>-1</sup> )	0	1.06	0.92	≤0.01	26.81	84
Cotton yield (Mg ha <sup>-1</sup> )	0	0.78	0.70	≤0.01	10.51	36
Daily emission of NH <sub>3</sub> (Kg ha <sup>-1</sup> d <sup>-1</sup> )	0	1.22	0.77	≤0.01	74.73	14
Yearly emission of NO <sub>3</sub> (Kg ha <sup>-1</sup> yr <sup>-1</sup> )	0	1.24	0.95	≤0.01	18.45	12



**Figure 1. Average daily soil temperature (0-5 cm) observed and simulated from 2013-2016**



**Figure 2. Average daily moisture content in the soil porosity (0-5 cm) observed and simulated from 2013-2016**

In addition to the conditioning rate, WFPS (%) also shows the availability of water (Paul, 2007). The best soil conditions for maximum N<sub>2</sub>O release occurred at temperatures ranging from 10 to 20 °C, with water filling 60–80% of the WFPS. In warm-dry and cold-wet conditions (beyond the optimum range), the N<sub>2</sub>O emission rate from the soil is very slow (Liang et al., 2018). As shown in Figure 2, the model prediction included temporal changes and soil moisture values measured at a depth of 0–5 cm in most cases, although there were some differences in some values. A linear correlation (R<sup>2</sup>) of 0.92 was obtained for the simulated values versus the observed mean daily moisture with a slope of 1.04 (P<0.01). The RMSE was calculated as 34.57% (Table 2).

### 3.2. Shoot biomass changes and yield

Simulated changes in shoot biomass for wheat and cotton were generally close to field observations (Figure

3). According to our findings, wheat shoot biomass was very low in winter, but it increased dramatically after the onset of spring, while it rose in cotton with a steady gradient during the plant's growth. This model successfully simulated this dynamic. The linear regression showed an  $R^2$  of 0.92 with a slope of 1.06 for the simulation of the cotton shoot biomass versus the observations ( $p < 0.01$ ) indicating a good agreement. An RMSE of 81.86% was obtained from the simulation results (Table 2). Figure 4 compares the simulated and

observed cotton yields in the three treatments between 2013 and 2017. The linear regression shows a good agreement between the simulated and observed yields with an  $R^2$  of 0.70, a slope of 0.78 ( $p < 0.01$ ), and an RMSE value of 51.5% (Table 2). The simulation results of the 5-year consecutive yields for the treatments (Figure 5) indicate that the tillage methods for cotton cultivation led to average yields of 3.26, 3.10, and 2.88 mg C ha<sup>-1</sup> in conventional, low, and no tillage treatments, respectively.

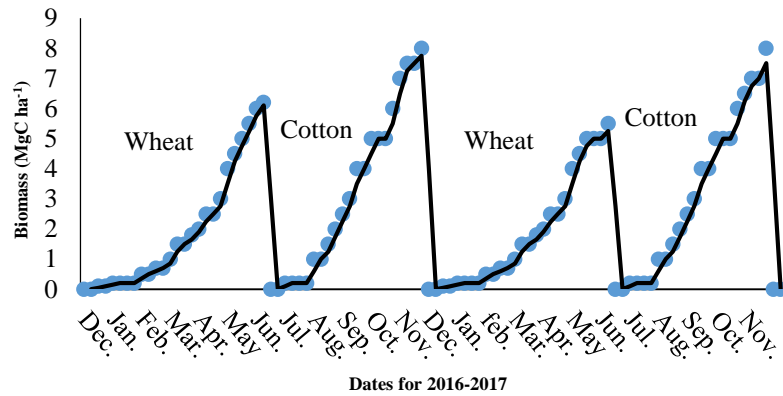


Figure 3. Average biomass observed and simulated in the wheat-cotton rotation from December 2016 to December 2017

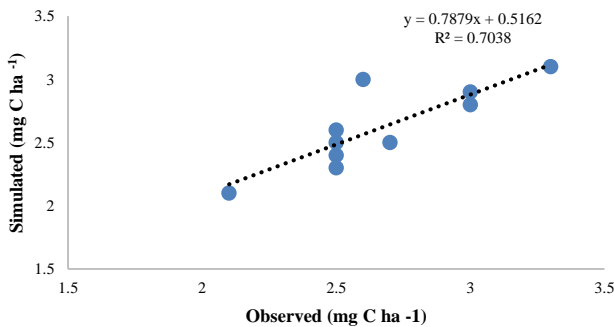


Figure 4. Correlation between observed and simulated average yields of cotton during the 5-year study

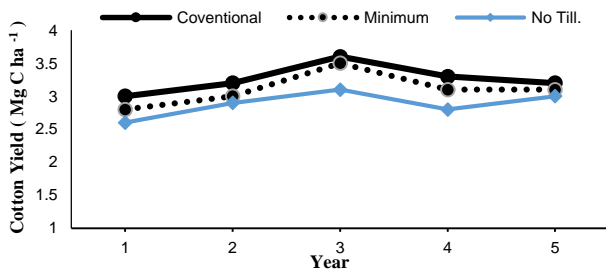


Figure 5. Comparison of cotton yields in different tillage treatments during five years of study

### 3.3. NH<sub>3</sub> emission from soil

Figure 6 displays the daily and cumulative NH<sub>3</sub> emissions from soil identified and simulated in the days following nitrogen fertilizer application in the conventional tillage treatment. According to the figure, the pattern of changes in the simulated fluxes corresponds to the observed values.

However model simulation showed an increasing peak flux on day 5 after fertilization, with a slight flux in the initial three days. Despite these differences, the model simulation obtained an NH<sub>3</sub> cumulative emission of 3.76 kg N ha<sup>-1</sup> resulting from fertilization operations, which was approximately 4% higher than the observed value (3.60 kg N ha<sup>-1</sup>). Linear regression of simulated against observed daily emission of NH<sub>3</sub> showed values of 0.77 and 1.22 ( $p < 0.01$ ) for  $R^2$  and slope, respectively, with an RMSE value of 74.73% (Table 2).

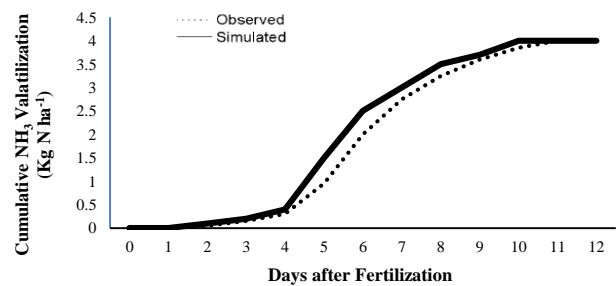


Figure 6. The observed and simulated daily (a) and cumulative (b) NH<sub>3</sub> emissions

### 3.4. N<sub>2</sub>O emission from soil

Figure 7 exhibits the simulated and observed emissions of N<sub>2</sub>O for the conventional and no -tillage treatments from October 2015 to October 2016. Daily N<sub>2</sub>O emissions were very variable every year. The highest values were usually observed after fertilization, irrigation (especially after fertilizer use), and heavy rainfall. CO<sub>2</sub> emission from the

soil depends on the soil temperature, while N<sub>2</sub>O emission shows a strong correlation with rainfall (Almaraz et al., 2009). Compared to the observed fluxes, the DNDC model generally showed the temporal pattern of N<sub>2</sub>O daily fluxes, although there were some differences in some N<sub>2</sub>O peak emissions.

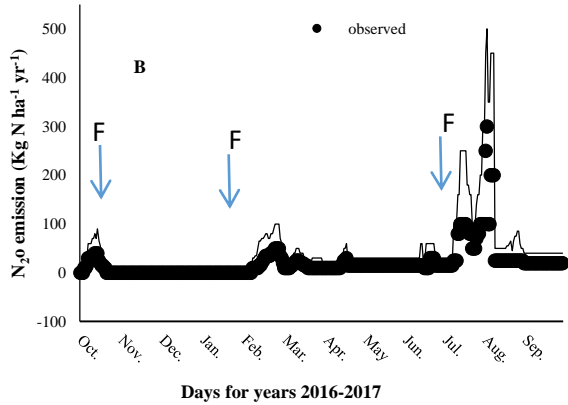


Figure 7. The observed and simulated daily N<sub>2</sub>O fluxes in no-tillage (A) and conventional tillage (B) treatments (F is the fertilization time).

In the three tillage treatments, the annual observed emission rate of N<sub>2</sub>O varied from 2.9 to 57.5 kg N ha<sup>-1</sup>, with an average of 3.70 kg (Figure 8). In accordance with the observations, model simulations for the annual N<sub>2</sub>O emission varied from 2.67 to 6.95 kg N ha<sup>-1</sup> with a mean value of 4.24 kg in different tillage treatments. A comparison of linear regression between simulated and observed N<sub>2</sub>O emissions in different treatments revealed an R<sup>2</sup> of 0.95 with a significant slope (p<0.01) of 1.24. An RMSE value of 19.45% was obtained for the simulated N<sub>2</sub>O emission (Table 2). According to the above results, the simulated and observed yearly N<sub>2</sub>O emissions were compatible and stable despite a wide range of management practices. This implies that the model simulation could accurately estimate the effects of various management practices on the emission of this gas.

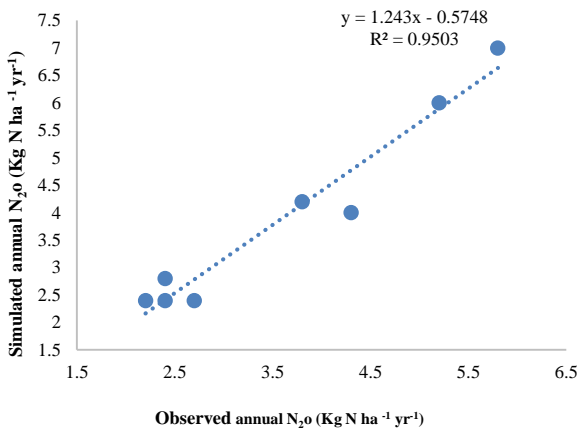


Figure 8. Annual cumulative distribution observed and simulated by N<sub>2</sub>O

The N<sub>2</sub>O emission rate in conventional tillage, with an annual average of 4.41%, was higher in all studied years than those in low and no tillage treatments, with annual averages of 2.00 and 2.14 kg N ha<sup>-1</sup>, respectively (Figure 9). The annual release of this gas in all the three treatments showed high variations, with a CV of 25–28% for N<sub>2</sub>O between different years.

The results of many studies are available on the use of conservational farming to reduce emissions of greenhouse gases from the soil, but these reports are very contradictory.

In a study, the use of conventional farming in maize fields had no effect on CO<sub>2</sub> and N<sub>2</sub>O emissions compared to conservational farming (Johnson and Barbour, 2010). In other studies, conventional agriculture reduced N<sub>2</sub>O emission from the soil (Dendooven et al., 2012; Singh et al., 2008), or the application of field conservational farming stimulated N<sub>2</sub>O emission through new N inputs in the plant biomass (Baggs et al., 2003; Harrison et al., 2002).

The conventional tillage led to the highest N<sub>2</sub>O emission from the soil in the cotton field (4.4 kg N ha<sup>-1</sup>) and zero tillage (2.1 kg N ha<sup>-1</sup>) (Figure 10). It seems that after rainfall and irrigation, the residues mixed with soil upon plowing in conventional tillage were degraded more rapidly, nitrogen released for denitrification and N<sub>2</sub>O production was more accessible than in the conservational tillage method. Under conservational farming, N<sub>2</sub>O emissions from the soil can decrease (Dendooven et al., 2012), increase (Baggs et al., 2003; Ussiri and Lal, 2009), or remain unchanged (Elmi et al., 2003; Jantalia et al., 2008; Omonode et al., 2007; Smith et al., 2011).

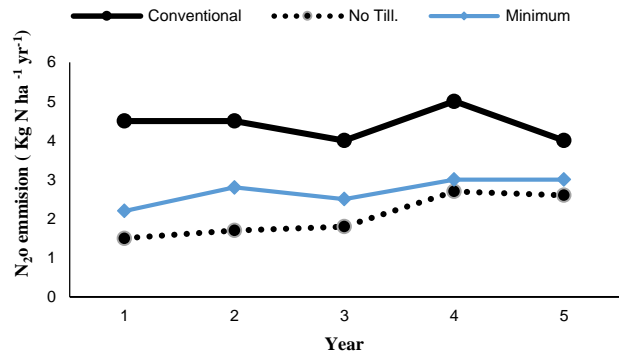


Figure 9. The simulated N<sub>2</sub>O emission rates in different tillage treatments in five years of study

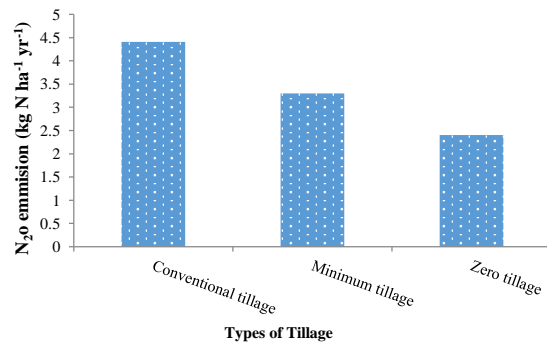


Figure 10. Annual N<sub>2</sub>O emissions in different methods of cotton field tillage

The results of some researchers were also consistent with ours. Spie et al. (2011) and Almaraz et al. (2009) reported significant differences between conventional and low tillage methods, so that reduction of plowing reduced N<sub>2</sub>O emissions in all studied scenarios. Grant et al. (2004) also reported that a change in farm management from conventional tillage to zero-plowing resulted in a decrease of about 17% in the mean weight of N<sub>2</sub>O emission during their entire 30-year study period in Canada.

#### 4. Conclusion

The DNDC model was tested and applied based on validation with limited variables of observations for agricultural systems in many countries (Giltrap et al., 2010). In this study, this model was validated through model simulation using measurements of soil moisture and temperature, crop growth and yield, and NH<sub>3</sub> and N<sub>2</sub>O emissions under different field tillage treatments in the wheat-cotton rotation. Despite the differences in daily simulations, validation results revealed that the model worked well in simulating the soil environment and the emission of gases.

In the Darab area of Fars Province, both surface dispersion of the base manure and tillage operations are carried out on a single day. The DNDC model runs daily. In the model, tillage occurs before fertilization, meaning that if fertilization and tillage are set up to be performed on a single day, the fertilizer is not incorporated into the soil. This will greatly reduce NH<sub>3</sub> emission and, as a result, other processes in the nitrogen cycle (Cai et al., 2003). To solve this problem, the soil tillage date can be simply determined one day after fertilization (as in this study). With continued rainfall and initial fertilizer application to cotton, N<sub>2</sub>O emissions were elevated in the spring. The emission was higher in the second than in the first year, and was greater in conventional than in conservational tillage, which seems to be due to the faster mineralization of residues in conventional than in conservational tillage treatment in which residues remain on the soil surface. The soil temperature in the no-tillage system was 1 °C lower on average than in the other tillage treatments. In all measurements, the no-tillage system had an average moisture content of 1-3% higher than conventional and low-tillage operations.

Overall, the results showed that tillage systems had significant effects on nitrogen gas emissions from cotton fields, with a reduction in the no-tillage method. It seems that preservation of wheat residues in conservational farming treatments (low and no tillage methods) in the cotton field could have a reducing effect on N<sub>2</sub>O and NH<sub>3</sub> emissions from the soil.

It can be concluded that in addition to reducing N<sub>2</sub>O and NH<sub>3</sub> pollutant emissions, conservation plowing in wheat-cotton rotation in Darab is economically justifiable due to fuel, time, production costs, machinery depreciation, soil erosion, human resources, and so on. Altogether, the present study considers tillage reduction as an essential element in improving the studied traits. As the no-tillage treatment in the cotton field was more favorable than the other treatments for cotton growing in wheat-cotton

rotation, it can be recommended in the same conditions as the present study.

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## Comparison of environmental impact assessment between irrigated and rainfed wheat using the life-cycle assessment method (LCA): The case of Khorramabad, Iran

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### ABSTRACT

The life-cycle assessment of two irrigated and rainfed wheat crops was investigated in this study. Tillage, planting, irrigation, fertilization, pesticide spraying, and harvesting are the basic stages of production for each crop throughout its life cycle. A farmer's questionnaire was used to collect farm data. The ecoinvent models compile emissions in their reports. The functional unit is designed to produce one ton of grain. Following the calculation, emissions from each stage of production in a triple environment (soil, water, and atmosphere) are logged. Following that, a life-cycle impact assessment, or LCIA, was carried out. The environmental effects were first estimated separately for each crop, followed by a comparison of the two crops. The calculated potential for each ton of irrigated wheat equals 860 Kg of CO<sub>2</sub> equivalent in the impact assessment of global warming, whereas it is 623 Kg for rainfed wheat. The production potential per ton for the eutrophication phenomenon is roughly equivalent for both systems: 2.625 equivalent Kg po<sub>4</sub> for irrigated wheat and 2.601 for rainfed wheat. The data from the long-term scenario show an increase in the potential for eutrophication in both crop productions. Meanwhile, the long-term effects of chemical fertilizer use on human health and aquatic and terrestrial ecosystems indicated a potential increase in their use. Following a data uncertainty analysis, it was determined that, with a confidence interval of 95 percent, details of the life-cycle assessment results could be applied to the farms under consideration. However, some of the environmental impacts in the uncertainty analysis overlap. The highest overlapping values are insufficient to call average farm results into question.

### 1. Introduction

Given the importance of environmental principles in production and agricultural processes, the most important option for justifying farmers is to introduce environmental impacts into production. Sustainability must be considered in terms of environmental conditions along with economic and social issues. Therefore, achieving sustainability or taking steps in this way involves taking into account the complexities of these three pillars and their interactions (Brentrup et al., 2004a; Hassani et al., 2016).

Life-cycle Assessment (LCA) is a standard method (ISO 14040, 2006) that is considered in this study. Applying LCA in the farm framework requires data on the inputs the farmer places

on the crops and on the outputs he receives, which measures the effects that will remain in place (Brentrup et al., 2004b). The impact assessment is done in accordance with the elaborated inventory, which ultimately can be interpreted by these panels as being useful to others and making decisions based on them (McGregor, 2002).

Determining a functional unit in the life-cycle analysis can be effective. A functional unit is a reference that interconnects the input and output of a produced crop. With such a unit, the researcher can compare different systems of different structures based on a common basis (Sonesson et al., 2010). The amount of inputs (including fossil fuels and mineral fertilizers), production and transfer of agricultural inputs (such as fertilizer

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production), and field operations (such as tillage and harvesting) for a functional unit should be determined (Khorramdel, 2011).

Many researchers have argued that LCA is a method for quantifying the environmental performance of products. The numerical ranking of this method enables the environmental performance of crops to be analyzed from the aspects of climate change, ozone depletion, acidification, eutrophication, depletion of energy sources, and other environmental impact groups. In this study model, inputs were collected based on data on the use of chemical fertilizers, machinery, fossil fuels, and other inputs for two crops (Smaielpoor et al., 2015).

The review of the life-cycle assessment should include the definition of the purpose and scope, the analysis of the inventory, the impact assessment, and the interpretation of the results. The interpretation of the results is associated with all the mandatory stages of the life-cycle. Therefore, the scientific and methodological determination of the various stages of the life cycle contributes to the emergence of a scientific interpretation. To set a life-cycle inventory according to the purpose and scope set, the attitude towards key issues is very important (Smaielpoor et al., 2015; Mir Haji et al., 2012; Khorramdel et al., 2015; Hosainzade et al., 2010). With regard to production inputs and how they can be used, they can be manipulated with scientific management to produce crops with the least environmental emissions. It should not be forgotten that natural resources will be exhausted, and any measures to preserve and conserve these resources will increase human healthy life on this planet.

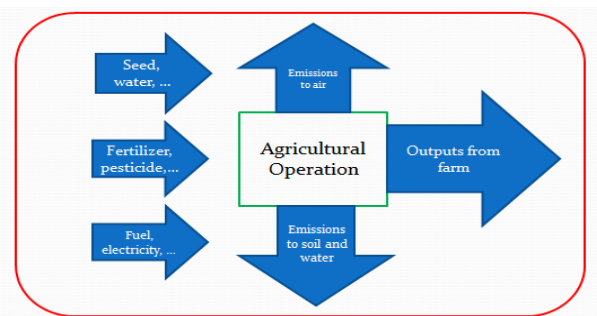


Figure 1. System boundary

This study tries to investigate the differences between the two wheat production methods in the study area. In the rainfed wheat production method, water and electrical energy are not consumed. Differences in intake and inputs are also seen. Amounts of labor, machinery and land occupation are different for a given functional unit. In spite of many differences in the production process, this paper examines the differences that may exist in environmental impacts.

Data uncertainty analysis has been performed to evaluate more precisely and clarify the differences between the two crops; many of the differences that are being examined through the average data may not be the same for all farms.

## 2. Materials and methods

### 2.1. Study area

The study area is Khorramabad in the semi-arid Lorestan

province of Iran. The city is located at 48°21' N, 32°3' E, at 1117 m above sea level. The average annual precipitation is 524 mm, and the average annual temperature is 17 °C. In this area, a lot of irrigation water is provided by wells. A mountainous region where steep land is abundant. Water resources suitable for dry farming have led farmers to use rainfed wheat on steep lands.

### 2.2. Life-cycle Assessment methodology

This part describes the steps of the LCA process and the data gathered for this study.

#### 2.2.1. Goal and scope

##### 2.2.1.1. Defining the goal of this study

The goal of this study was to evaluate the environmental effects of irrigated and rainfed wheat crops in the study area. The purpose of this study is to determine how the damage caused by the cultivation of these crops will enter the environment.

Identifying and simulating environmental impacts in the study area will help farmers to understand and have a better perception of reducing environmental impacts.

##### 2.2.1.2. System boundary

The boundary defined for this experiment is the farm framework. Data information is collected in a farm context. A flowchart is presented to better understand the data collection process and the system boundary (Figure 1).

Flowchart information includes an overview of inputs, outputs, agricultural operations, and emissions. All components of the LCA will be defined in this scope, and the final stage of the interpretation will be presented in this scope.

##### 2.2.1.3. Functional unit

Functional unit is a reference used to compare different systems based on a common structure (Wiedemann and Mcgahan, 2011).

The selected functional unit was one ton of harvested grain. All stages of agricultural operations, inputs, and emissions are calculated for this functional unit.

#### 2.2.2. Life-cycle inventory (LCI)

In the inventory stage, all the inputs of the cropping system are accurately described, as are all the outputs and emissions to the triple environments. Different stages have been defined for the production of irrigated and rainfed wheat. Information about the various stages of production of crops is given in Table 1.

#### 2.2.3. Impact assessment

In the present research, the main goal is to investigate the environmental impacts of the crops, so the impact chains in relation to the impact assessment have been selected along with the European series of guidelines. In this study, the CML Recipe command was used for evaluation. These environmental impacts are first examined separately during the life-cycle assessment of the two crops and then compared.

Each impact assessment has an abbreviation and an equivalent unit that is available in Table 2 (Goedkoop et al., 2008, Pre consultants, 2003, Ahmadi and Ghasempour, 2016).

**Table 1. Agricultural operations defined, according to relevant inputs**

Agricultural operation	Tools and materials	Other information
Tillage	Plough, rotary cultivator, tractor made is Massey Ferguson 285 (75 hp), diesel	Tractor weight (2746 kg), plough (360 kg), rotary cultivator (600 kg)
Planting	Planter, tractor (75 hp), diesel, seed, pesticide for disinfection	Planter for wheat (680 kg) for non-irr wheat (700 kg)
Irrigation	Water pump (40 kw), electrical energy, labor	Just for wheat, water use in well (865.67 m <sup>3</sup> )
Fertilization	Urea, triple super phosphate, Potassium sulfate, tractor (75 hp), sprayer, diesel	Fertilizer sprayer (350 kg)
Plant protection	pesticide, sprayer, tractor (75 hp),	Sprayer (380 kg) and Vol. (400 lit)
Harvest	Combine, truck, diesel	Combine (105 hp) and (2600 kg), Tank vol. (2700 lit)

Farm inputs in this system are environmental inputs (water, carbon dioxide in the air, land occupation, etc.), Technosphere inputs (tools, materials, machinery, etc.), and energy inputs (electricity, fossil fuels, etc.).

### 2.2.4. Interpretation

There are several basic elements in the life-cycle interpretation that, in their view, can provide an acceptable interpretation by the researcher. These elements can be categorized as follows:

- Identify important issues based on the results of the life-cycle inventory in process and the assessment of this inventory in the overall life-cycle assessment.
- An assessment that considers completeness, sensitivity, and consistency.
- Finally, make conclusions, limitations, and recommendations.

### 2.3. Investigating uncertainty of data

Due to the fact that the test data is collected from different farms, it's not possible to announce the results with certainty for all of the farms. Some farms receive more inputs than others, and due to this difference, environmental damage can also be different. In this study, an uncertainty analysis was performed using the Monte Carlo method to compare the two systems. The confidence interval in the uncertainty analysis is 95%. The distribution used in this analysis is the Monte Carlo method.

### 2.4. Source of emission data

The calculation of emissions is intended for the three environmental compartments: soil, water, and atmosphere. A summary of the types of emissions and their sources is given in Table 2.

**Table 2. Impact scores - equivalent units and specifications**

Impact assessment	Unit	comment
Natural resources depletion, abiotic (AD)	kg Sb equivalent.	This potential consists in the consumption of renewable and non-renewable resources.
Abiotic depletion, fossil fuels (ADF)	MJ	Exploitation of fossil fuels, mineral resource and also the potential of fossil resource depletion.
Global warming potential (GWP)	Kg CO2 equivalent	Potential share of one material in greenhouse emissions impact.
Ozone layer depletion (ODP)	kg CFC-11 equivalent	Value of ozone layer destruction, which is mainly created by hydrocarbons including carbon, chlorine and fluorine.
Human toxicity potential (HTP)	kg 1,4-DB equivalent	Damage potential of one unit of released chemical material to the environment based on the toxicity of a combination and its potential of consumption dose.
Terrestrial eco-toxicity (TE)	kg 1,4-DB equivalent	Emissions of toxic substances to soil.
Fresh-water aquatic eco-toxicity (FEW)	kg 1,4-DB equivalent	Emissions of toxic substances to fresh water.
Marine eco-toxicity (ME)	kg 1,4-DB equivalent	Refers to impacts of toxic substances on marine ecosystems.
Photochemical oxidation (PO)	kg C2H4	The potential has is expressed as the creation of the one capacity of ozone of volatile organic material for ozone production.
Acidification (AC)	kg SO2 equivalent	The potential shows the acidification impact of SO <sub>2</sub> . Another material that has been recognized as acidification, is nitrogen oxide and ammonium. Also the impact of SO <sub>x</sub> is similar to SO <sub>2</sub>
Eutrophication (EU)	kg PO <sub>4</sub> <sup>-2</sup> equivalent	The potential was used based on PO <sub>4</sub> <sup>-2</sup> , another emission of eutrophication were nitrogen oxidation N <sub>2</sub> O and ammonium NH <sub>4</sub> <sup>+</sup>

**Table 3. Data sources for emission to the triple environment**

Compartment	Emissions	Data sources
Atmosphere	NH <sub>3</sub> , CO <sub>2</sub> , N <sub>2</sub> O, NO <sub>x</sub> , SO <sub>2</sub> , CH <sub>4</sub> , CO, etc.	Bengona et al., 2015. Nemecek and Kagi, 2007. Nemecek and Schntzer, 2011c. Agrommon, 2009.
Water	Nitrate, phosphate, cadmium, lead, zinc, etc.	Bengona et al., 2015. Nemecek and Kagi, 2007. Nemecek and Schntzer, 2011b. The emission model SALCA-P & SALCA-NO <sub>3</sub> , 2006.
Soil	Cadmium, lead, zinc, etc.	Nemecek and Kagi 2007. Nemecek and Schntzer, 2011a. Robert and Stauffer, 1996.

### 2.5. Database and software

The global database used in this study is the ecoinvent database. The database is reviewed and updated over time, with the latest version being version 3 at the time of the current research. The ecoinvent 3 has more different models and

methods than the ecoinvent 2. Documents related to this extension and the changes are available on the site. The SimaPro software used for the life-cycle assessment method describes the software version and specifications below. SimaPro 8, report version V3, language: English.

### 3. Results and discussion

#### 3.1. Irrigated wheat life cycle assessment

Rainfed wheat is also a cereal for cultivation in the study area. Therefore, in terms of cultivation, it follows a completely observable process with wheat. Most of the items used are the same, and the difference in the production stages is due to the lack of irrigation in rainfed wheat.

The results of the evaluation of the wheat life-cycle assessment production are in accordance with Table 3. The various stages of wheat production are tillage planting, fertilizing, irrigation, pesticide spraying, and harvesting. Figure 2 shows the percentage of production steps for each potential impact.

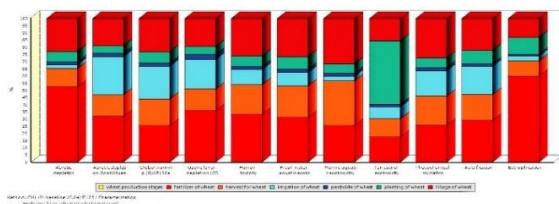


Figure 2. Contribution of irrigated wheat production steps to midpoint impact indicators

Table 4. Environmental damage resulting from the production of one ton irrigated wheat

Impact assessment	Unit	Amount
Abiotic depletion	Kg Sb eq	0.005815
Abiotic depletion (fossil fuels)	Mj	11709.96
Global warming (GWP100a)	Kg CO <sub>2</sub> eq	860.6032
Ozone layer depletion	Kg CFC eq	0.000101
Human toxicity	Kg 1,4-DB eq	445.1575
Fresh water aquatic eco-toxicity	Kg 1,4-DB eq	207.227
Marine aquatic eco-toxicity	Kg 1,4-DB eq	1408588
Terrestrial eco-toxicity	Kg 1,4-DB eq	3.452
Photochemical oxidation	Kg C <sub>2</sub> H <sub>4</sub> eq	0.2707
Acidification	Kg so <sub>2</sub> eq	5.659
Eutrophication	Kg Po <sub>4</sub> eq	2.625

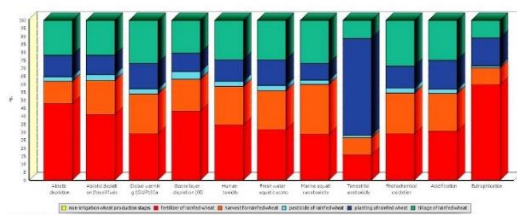


Figure 3. Contribution of rainfed wheat production steps to midpoint impact indicators

#### 3.3. Comparison between both systems

To compare the two systems, many items are considered. Due to the fact that there is no irrigation in rainfed wheat, the first five common stages were surveyed (Figure 4).

The presence of similar inputs in the two systems makes comparisons easier. Initially, environmental inputs are required. With the yield being lower in the rainfed system, land occupancy is greater, and inputs per ton should be also increased.

The next issue to be taken into consideration is the difference in technological inputs required to produce one ton of grain. When more land is occupied for production,

According to the results of the data analysis, the most impactful steps for abiotic and fossil fuel depletion are due to fertilization and to the use of chemical fertilizers. The greatest impact on fertilization operations can be seen in the potential impact of eutrophication. Tillage operations have significant effects on the majority of impact assessments.

#### 3.2. Rainfed wheat life-cycle assessment

The results of the rainfed wheat are shown in Table 4. Different stages of rainfed wheat production include tillage, planting, fertilizing, pesticide spraying, and harvesting. Fewer inputs have been used in rainfed wheat. In Figure 3, the percentage of production stages is shown for different environmental impacts. Due to the lack of irrigation in rainfed wheat, the planting process has the greatest impact on the environmental damage of the terrestrial ecosystem.

For the other stages, the same tendencies as with irrigated wheat are observed (Figure 3 and Table 5).

The risk of increasing nitrate levels in groundwater and the potential for eutrophication in the fall and winter months when rainfall is often or always more than the absorption of plants (Stauffer et al., 2001).

mechanization also increases. As a result of fossil fuels, machinery depreciation and environmental emissions increase. Figure 4 highlights the difference between the producing stages for one ton of wheat in irrigated and rainfed systems.

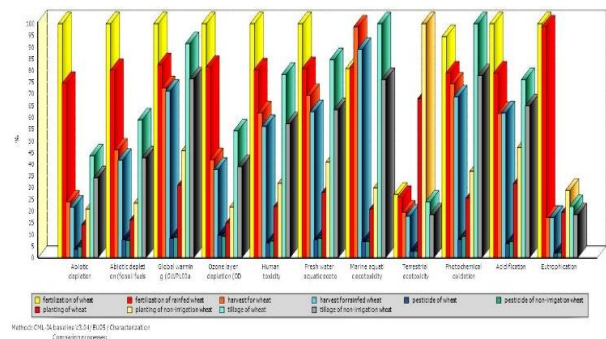


Figure 4. Comparison of the stages of production of irrigated and rainfed wheat

(normalized by the most impacting stage for each potential impact)

From the diagram, it is quite clear that the use of chemical fertilizers in the two models of planting has the

greatest effect on eutrophication potential. Nevertheless, the effect of fertilizer use in the irrigated system is slightly higher than that of the rainfed one. Eutrophication potential is calculated on the basis of PO<sub>4</sub>. among other emissions affecting eutrophication, nitrogen oxides (N<sub>2</sub>O) and ammonium NH<sub>4</sub><sup>+</sup> can be mentioned. Eutrophication is the unintentional increase in the production of biomass in terrestrial and aquatic ecosystems due to the entry of nutrients, which can change the composition of plant species. Eutrophication is particularly dangerous in surface waters, as it can exacerbate the growth of algae and lead to the loss of life in ponds and lakes (Brentrup et al., 2004a).

According to Figure 4, the planting process has the greatest impact on the toxicity of the terrestrial ecosystem. The effect of rainfed wheat planting is greater on terrestrial ecosystem eco-toxicity. The probability of these results may be due to the phenomenon of occupied land. Farmers choose land and farming according to their assets and financial level. Drought fields in Khorrabad are located on more slopes, except for the farmer's assets. Irrigated agriculture is not possible on these lands or financially, it does not meet the costs of the farmer (Hassani and Ramroodi, 2017). Therefore, land occupation is higher for a ton of rainfed wheat and will cause a wider range of damage to the terrestrial ecosystem.

The use of chemical fertilizers has a significant impact on abiotic depletion, and rainfed wheat is more effective in terms of abiotic resource and fossil fuel impacts. In the impact assessment of marine aquatic eco-toxicity, the stage of tillage and harvesting is very influential. Compared to the two systems, they have a significantly higher effect on the irrigated one.

In terms of global warming potential, the fertilization, tillage, and harvesting stages have the most effect, respectively. Similarly, a lesser degree of ozone depletion is also observed. In two stages of fertilization and tillage, the impact of producing one ton of wheat is more than one ton of rainfed wheat in terms of global warming and the ozone depletion potential. The results indicate a great similarity in the effect of the harvesting stage for the two cropping systems. Other comparisons between the stages of production and the impact assessment are clearly evident in Figure 4.

### 3.4. Uncertainty analysis

After evaluating the test data using the uncertainty method, the results are shown to have a greater difference than the life-cycle assessment comparison. The method chosen to assess uncertainty is the use of the Monte Carlo distribution. The assumed confidence interval is 95%. Chart uncertainty comparing wheat and non-irrigated wheat is available in Figure 5.

The results presented in the chart above show uncertainty comparisons of 11 environmental impacts for both systems. The results of this analysis show that 95% of the environmental impacts of irrigated wheat are greater than or equal to rainfed wheat, and in all environmental impacts, this is a priority.

It should be noted that the percentage of cases where rainfed impacts are higher than irrigated impacts is not negligible. Therefore, there may be potential for further

damage to the environment in some rainfed wheat fields. Accordingly, the environmental effects of eutrophication, global warming, acidification, abiotic depletion, and photochemical oxidation cause the highest occurrence of this inversion.

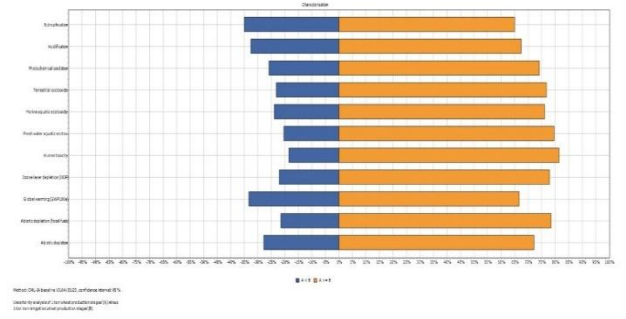


Figure 5. Results of uncertainty assessment of environmental impacts for one ton of wheat. A: irrigated wheat, B: non irrigated wheat. 95% confidence interval

To better understand this result, the results of the uncertainty caused by the phenomenon of eutrophication are expanded. Figure 6 shows the comparison of the two systems in this environmental impact.

In the chart above, the data uncertainty range is quite clear. The values are similar to the mean in the graph, around the red dot. Due to the increase in the effect of eutrophication in A (irrigated), many datasets with high similarity and even more in the range of product B (rainfed) are seen. Therefore, it is not correct to say with certainty that the potential for eutrophication of one ton of irrigated wheat is always higher than one ton of rainfed wheat. In the rest of the environmental impacts mentioned, this overlap is much lower, and so producing irrigated wheat has more potential to produce environmental damage.

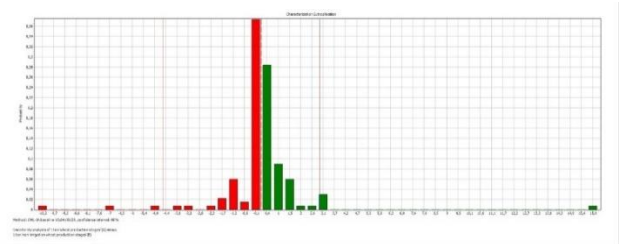


Figure 6. Comparison of the uncertainty of eutrophication in the production of one ton of irrigated and rainfed wheat. 95% confidence interval. Red: irrigated system data, Green: rainfed system data

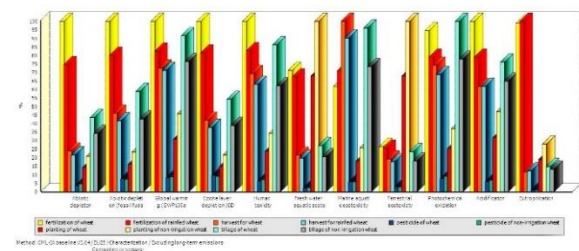


Figure 7. Comparison long-term emissions of irrigated and rainfed wheat production

### 3.5. Long-term emissions

One of the capabilities of the SimaPro software is its long-term environmental impact calculations. Looking at Figure 7 and comparing it with Figure 4, the differences created over time are specific. The severity of the difference in impact assessment groups will be different, and in some cases, there will be no significant difference.

In this assessment over the long-term, the effects of fertilization on the eutrophication indicator have increased for rainfed wheat. The next step is to increase the impact of the planting process on the freshwater aquatic eco-toxicity, which increases over time. Initially, it had the greatest impact on marine aquatic eco-toxicity, but in the long-term, it also affects freshwater aquatic eco-toxicity.

An important issue is the increased environmental damage caused by the increased effects of human toxicity during the tillage and harvesting stages. The emission of these two processes will, in the long-term, increase the severity of harm to human societies. In the impact assessment of human toxicity, the increase in fertilization rate in rainfed wheat is also known. Indeed increasing the number of emissions related to the tillage process and harvesting in the two systems in the long-term will affect human toxicity more.

## 4. Conclusions

### 4.1. Life-cycle assessment results

The global food production process and the emissions of agricultural processes intensify the effects of eutrophication and acidification phenomena and increase global warming and climate change (Saarinen et al., 2012). In recent years, researchers have increasingly used life-cycle assessment to identify environmental impacts so that they may be able to come up with ways to produce beneficial food (Notarnicola et al., 2012).

Actions that prevent soil erosion are effective in reducing environmental impacts. Like green manure, due to the reduction of soil erosion, nutrients enter the soil and eventually lead to less use of chemical fertilizers (LBL et al., 2000).

The production of food through agricultural processes involves pollutant emissions into the environment. For example, ammonia (NH<sub>3</sub>) can be released, accounting for about 93% of the agricultural process (Thoni et al., 2007), methane (CH<sub>4</sub>), nitrate (NO<sub>3</sub><sup>-</sup>), etc. (Nemecek and Kagi, 2007). Ammonium (NH<sub>4</sub><sup>+</sup>) in the chemical fertilizers used in the agricultural process can easily be converted into ammonia (NH<sub>3</sub>) and released into the air. Agriculture has been evaluated in Switzerland as the largest ammonia production process (Thoni et al., 2007, Nemecek and Schntzer, 2012).

The major findings of this project are the following:

- 1- The difference between the global warming potential of irrigated and rainfed wheat is probably due to irrigation in rainfed wheat. This difference could be due to the lack of electricity in the energy section of the life-cycle inventory and the lack of water in the environmental inputs set in the life-cycle inventory.
- 2- Rainfed wheat may have even higher global warming and greenhouse gas emissions than wheat in the event of irrigation because the land used to produce rainfed wheat is steeper (from the information collected in the field questionnaire) and

because the functioning of the machinery to produce one ton of cereal is longer and uses more fossil fuel.

3- Considering emissions affect environmental impacts in the long term, most research findings are based on one year of production. Over time, the accumulation of emissions from previous years can make the environmental situation more unpredictable.

4- According to the scenario, the impact of long-term emissions on the environmental impact of human health is quite evident. It should be noted that other environmental impacts of aquatic and terrestrial ecosystems are also affected.

5- Considering the small difference in environmental potential between irrigated wheat and rainfed wheat, the irrigated system may be more suitable in the studied area. Due to the weather conditions in this upland, more energy and hours of operation are needed for rainfed wheat.

Irrigated wheat on flat land and suitable conditions may have a lower environmental impact. According to the findings, the land selection is important for different agricultural processes.

Regarding mechanization and field operations, topographic conditions should also be considered in land use planning. Government infrastructure, especially in third-world countries, is important for supporting farmers. Changing the attitude of farmers towards the environment and the pursuit of nature-friendly can help achieve sustainability indicators.

### 4.2. Results of uncertainty analysis

After uncertainty analysis of the data, it became clear that, with a confidence interval of 95%, details of the results of the life-cycle assessment could be applied to the studied farms. Although there are some cases with higher impacts for rainfed wheat, the ratio between the cases where the irrigated system has more impact (relatively to the rainfed system) confirms the results of the average farms. The most critical case appears to have eutrophication potential.

The results of the inventory analysis of both cropping systems showed that the fertilization operation had the highest effect on this environmental indicator. Fertilization operations are planned according to the farm area. Therefore, products with a lower yield will receive more inputs to produce a functional unit.

**Table 5. Environmental damage resulting from the production of one ton rainfed wheat**

Impact assessment	Unit	Amount
Abiotic depletion	Kg sb eq	0.004619
Abiotic depletion (fossil fuels)	Mj	7291.318
Global warming (GWP100a)	Kg CO <sub>2</sub> eq	620.2596
Ozone layer depletion	Kg CFC eq	6.83E-5
Human toxicity	Kg 1,4-DB eq	339.3906
Fresh water aquatic eco-toxicity	Kg 1,4-DB eq	164.2487
Marine aquatic eco-toxicity	Kg 1,4-DB eq	1252330
Terrestrial eco-toxicity	Kg 1,4-DB eq	3.762
Photochemical oxidation	Kg C <sub>2</sub> H <sub>4</sub> eq	0.1976
Acidification	Kg so <sub>2</sub> eq	4.067
Eutrophication	Kg po <sub>4</sub> eq	2.542

### 4.3. Conclusion

The activity of farmers as food producers should be appreciated. Paying attention to this hardworking class and trying to modify their production processes is very beneficial for the future of food and the resources of the planet. In order

for humans to live longer on Earth, they need to understand the principles of the state of resources and modify their pattern of consumption.

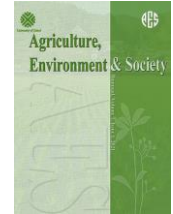
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## Agrophysiological of barley genotypes responses to zinc fertilization and water saline irrigation

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### ABSTRACT

Salinity is one of the most important abiotic stresses because it causes zinc to precipitate in an unusable form for plants and is influenced by saline-calcareous soils. This experiment was carried out in a strip split block design with three replications at the Esfahan Rodasht Drainage and Salinity Research Station to investigate the effects of agrophysiological responses of barley genotypes to zinc fertilization and water saline irrigation. As vertical factors, water irrigation quality at three levels, 2, 10, and 18, dS/m, were used. Fertilizer application included Nano zinc-oxide, zinc-chelate, a mixture of Nano zinc-oxide and zinc-chelate, and water as a control. Within vertical factors, three different barley genotypes are arranged, including Morocco (moderate semi-sensitive), Nosrat (moderate tolerant), and Khatam (tolerant). The results showed that the application of Zn-chelate fertilizer resulted in the highest grain yield, K<sup>+</sup> concentration, and K<sup>+</sup>/Na<sup>+</sup> ratio in shoots. In Khatam, stomatal conductance (gs), the maximum quantum efficiency of PSII (Fv/Fm), K<sup>+</sup> and Zn<sup>2+</sup> concentrations, and the K<sup>+</sup>/Na<sup>+</sup> ratio were all higher than in Morocco. In comparison to Morocco, Khatam had lower Na<sup>+</sup> and Ca<sup>2+</sup>+Na<sup>+</sup> contents. Furthermore, as salinity stress increased, all barley genotypes showed a decreasing trend in K<sup>+</sup> content and the K<sup>+</sup>/Na<sup>+</sup> ratio in shoots.

### 1. Introduction

Population rise and salinity in the world have increased water requirements. The use of gray water (drainage and recycled water) for production has been suggested as a solution to save high-quality water. However, the application of gray water causes salinity tension (Zhuo and Hoekstra, 2017).

Salinity is a serious threat to agricultural sustainability because over 800 million hectares of land throughout the world are salt-affected (Munns, 2005). Salinity can hamper plant growth and yield in two ways (Arzani and Ashraf, 2017): firstly, high concentrations of salts in the soil disrupt the capacity of roots to extract water (Mahlooji et al., 2018) and decrease the root-zone osmotic potential (Ashraf et al., 2008), and secondly, high concentrations of salts within the plant itself can be toxic (Munns, 2002; Munns and Tester, 2008) and later cause ion toxicity (Tabatabaei and

Ehsanzadeh, 2016), particularly through the accumulation of Na<sup>+</sup> ions (James et al., 2008).

Understanding salt-tolerant responses and mechanisms is imperative for crop improvement in salt regions. Plants' responses to salt stress are an expression of the physiological changes that occur in the plant to overcome the environmental stress imposed by salinity (Venkateswarlu et al., 2012). The initial and most dramatic response of plants, when exposed to salt conditions, is a decrease in stomatal conductivity. Stomatal conductance (gs) and resistance are reduced by salinity in the root zone. The stomatal conductance is initially affected by salinity due to water relations and subsequently due to the synthesis of ABA (Fricke et al., 2004; Fricke et al., 2006; Mahlooji et al., 2014). There is a signal from root to stomata when the plants are exposed to water stress (Davies et al., 2005) or when the plants are exposed to saline soil with a high concentration of salts (Termaat et al., 1985).

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Some reports of salinity effects on the photochemical efficiency of Photosystem II (PSII) of different plant organs, tissues, and cell preparations are limited and conflicting. Some researchers have demonstrated that salt stress inhibits PSII activity (Hichem et al., 2009), whereas others have indicated that salt stress has no effect on PSII (Lu et al., 2003a, b; Demiral and Türkan, 2006). It has been reported that mild-salinity levels do not induce sustained photodamage to PSII as revealed by unvaried Fv/Fm (photosynthetic activity or maximal PSII photochemistry) in plants (Baker and Rosenqvist, 2004; Naumann et al., 2007), even if leaf gas exchanges are reduced. In the first stages of salinity stress, stomatal conductance decreases and, consequently, CO<sub>2</sub> fixation leaves PSII unaffected (Baker and Rosenqvist, 2004). However, some studies have shown changes in the Fv/Fm ratio after dark-adaptation of the leaf as a result of salinity (Lee et al., 2004).

Differences in ion partitioning and the maintenance of higher nutrients such as nitrogen, phosphorus, potassium, and calcium to Na<sup>+</sup> ratios, especially in young growing and recently expanded tissues of barley, would appear to be important mechanisms contributing to the improved salt tolerance (Wei et al., 2003). Among glycophytic plants, barley is one of the most salinity-stress tolerant species, with an 8 dS/m degree of salt tolerance (Pessarakli, 1995). Salt stress causes a nutritional imbalance through lowering phosphorus (Evelin et al., 2009), potassium (Khosh Kholgh Sima et al., 2009), nitrate (Aslam et al., 1984), calcium (Hu and Schmidhalter, 2005), and zinc (Karimian and Moafpouryan, 1999; Khoshgoftarmanesh et al., 2004) absorption.

Plant mineral element nutrition is affected by salinity. Pearson and Rengel (1994) investigated Zn<sup>2+</sup> and Fe<sup>2+</sup> transport into remobilization conditions from leaves and found that Zn<sup>2+</sup> showed good remobilization. Soil salinity is also associated with zinc efficiency in alkaline conditions (Khoshgoftar et al., 2006). By reducing the amount of soil moisture in saline soil, Zn<sup>2+</sup> and Fe<sup>2+</sup> in the soil solution are reduced in mobility. Application of Zn fertilizers is a common practice to correct Zn<sup>2+</sup> deficiency. Zn<sup>2+</sup> is an essential micronutrient that is deficient in many regions worldwide, such as in the calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh et al., 2004). Zinc application increased corn, wheat, and soybean yields (Hemantaranjan and Gray, 1988). However, soil application of Zn<sup>2+</sup> has not been very successful under furrow irrigation. Zinc deficiency in plants grown in calcareous soils can be fairly corrected by the application of inorganic zinc salts such as ZnSO<sub>4</sub> (Sielsepour, 2006). El-Fouly et al. (2010) suggest that in saline soils, some elements such as magnesium, calcium, and sodium have antagonistic effects on micronutrients uptake by the roots. The high pH and CaCO<sub>3</sub> content of these soils are usually considered the reasons for the low availability of Zn (Karimian and Moafpouryan, 1999; Havlin et al., 2005; Mahlooji and Pessarakli, 2017). Most Zn<sup>2+</sup> deficiencies can be corrected with foliar zinc application (Christensen and Peacock, 2000). The deficient elements in plants can be sprayed with a solution to compensate for their deficiencies

(Cakmak, 2008). Applying the micronutrients could restore the negative effect of salinity on dry weight and nutrient uptake (El-Fouly et al., 2010). Many studies suggest that micronutrient fertilizers could increase plant resistance to environmental stresses such as drought and salinity (Shahlaby et al., 1993). Khoshgoftar et al. (2006) found that the application of Zn<sup>2+</sup> had a positive effect on the salt tolerance of wheat. In Iran, the increasing importance of Zn fertilizers and the use of water in saline soil to suppress regular salinity have been demonstrated as a requirement for studies of their combined effects on crop growth. Limited research has been conducted on the effects of zinc foliar application and salt resistance. Salinizing with NaCl has been shown to decrease free Zn<sup>2+</sup> concentrations in soil solutions (Khoshgoftar et al., 2004). Improving the salt resistance of crop plants is a major focus of agricultural research. There are a few studies where links have been established between concentrations of Zn<sup>2+</sup> in different barley genotypes under saline soils.

The purpose of this study was to investigate the effects of salinity and zinc fertilization on the physiological responses of barley genotypes. Furthermore, Zn<sup>2+</sup> fertilizations (in the forms of EDTA and Nano-ZnO) were tested as a means of reducing salinity stress.

## **2. Materials and methods**

### **2.1. Plant materials and treatments**

In this experiment, the effects of irrigation water salinity stress and zinc fertilizer applications on barley genotypes were evaluated. In order to find out the changes in agrophysiological properties, such as yield, stomatal conductance, chlorophyll fluorescence, and nutrient content of three barley genotypes, Morocco (salt-sensitive), Nosrat (semi-salt tolerant), and Khatam (salt-tolerant). This experiment was conducted in a strip-split-plot design with three replications at Esfahan Rodasht Drainage and Salinity Research Station (32° 30' N, 52° 09' E) in two cropping seasons (2012-14). Three irrigation water qualities, including W1=2 dS/m (low salinity as a check), W2=10 dS/m (common salinity in the region), and W3=18 dS/m (high salinity), were evaluated as vertical strip factors. The horizontal factors were four levels of foliar application, including Nano zinc-oxide, Zn-chelate, a mixture of Nano zinc-oxide and Zn-chelate, and water application as a check. The application rates of Nano-ZnO and Zn-chelate were 100 and 1000 grams per hectare, respectively. Soil characteristics and chemical analysis of irrigation water quality have been shown in Table 1.

Barley genotypes were planted in 1.2×4 m plots in November. The seeding rate was 450 seeds per square meter in June. Samples for grain yield are harvested and weighed at physiological maturity stage. Phenological stages were determined according to the method suggested by Zadoks et al. (1974).

### **2.2. Nutrient concentration**

Digestion apparatus methods determined the nutrient concentration in the shoots. The rates of N, P, and K are determined by the Auto Analyzer (Quikchem IC+FIA 8000

Series) and the rate of Na<sup>+</sup> is determined by the Atomic Absorption Spectrometer (Perkin Elmer Model 3110 USA) (Bauder et al., 2014).

### 2.3. Physiological parameters

**Stomatal conductance:** Direct measurements of photosynthesis through gas exchange rate were performed by an infrared gas analyzer (IRGA), which measures the

carbon dioxide flux within a sealed chamber containing a leaf sample.

At grain filling, stomatal conductance was determined by using a portable photosynthetic system IRGA (Model:LCA-4, USA) on intact plants (abaxial surface of the mid portion of flag leaf) between 10:00 and 14:00 hours, and light intensity was set at 1200-1400 μmol/m<sup>2</sup>s with an infra-red/blue light source (Fischer et al., 1998).

**Table 1. Selected physico-chemical properties of the soil before planting and three levels of water irrigation quality**

Soil characteristic	Amount	Water characteristics	Saline water		
			W <sub>1</sub> =2 (dS/m)	W <sub>2</sub> =10 (dS/m)	W <sub>3</sub> =18 (dS/m)
pH	7.7	pH	7.7	8.1	7.6
Electrical conductivity (dS/m)	13	Electrical conductivity(dS/m)	1.4	9.7	17.8
Available K (mg kg <sup>-1</sup> )	340	So <sub>4</sub> <sup>2-</sup> (meq/lit)	0.8	26.9	172.3
Available Zn (mg kg <sup>-1</sup> )	0.72	HCO <sub>3</sub> <sup>-</sup> (meq/lit)	2.0	5.7	6.4
Available Fe (mg kg <sup>-1</sup> )	5.54	Cl <sup>-</sup> (meq/lit)	1.4	60	111
Available Na (meq/lit)	79.1	Na (meq/lit)	1.5	47.8	99.3
Available Ca+Mg (meq/lit)	60	Ca+Mg (meq/lit)	2.6	44	72

**Chlorophyll Fluorescence:** Chlorophyll fluorescence was measured with a leaf promoter (Handy OS1-FL, USA) on intact plants (abaxial surface of the mid portion of the flag leaf) between 10:00 and 14:00 hours at the heading stage. The leaves were maintained in darkness by clips for 20 min before taking the data on chlorophyll fluorescence (Bake and Rosengvist, 2004; Li et al., 2009).

The chlorophyll fluorescence characters included the minimal fluorescence level from dark-adapted leaves (F<sub>0</sub>), maximal fluorescence level from dark-adapted leaves (F<sub>m</sub>), variable fluorescence level (F<sub>v</sub>), the maximum quantum efficiency of PSII photochemistry (F<sub>v</sub>/F<sub>m</sub>= (F<sub>m</sub>-F<sub>0</sub>)/F<sub>m</sub>). For each treatment, the chlorophyll fluorescence of 3 individual leaves was measured.

### 2.4. Data 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 ≤ 0.05).

### 3. Results

Rainfall in the first year (2012-13), second year (2013-14), and the average (2002-2014) was 149.8, 97.2, and 93.5 mm, respectively. Rainfall in the first year of the study was 60.2% (56.3 mm) greater than the average mid-term rainfall. In May of the first year (the time of heading and grain filling stages), the rainfall was three times that of normal years, and the temperature was 7 °C lower than normal years.

**Table 2. Effects of water quality, fertilizer application and genotypes on grain yield, stomatal conductance (gs), Fv/Fm (maximum quantum efficiency) on shoot nutrient contents (Fe, Zn)**

Treatments	Grain yield		Stomatal conductance		Fv/Fm		Fe		Zn	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
<b>Quality(dS/m)</b>										
2	6006.30a	3123.26a	0.505a	0.211a	0.799a	0.76a	204.83a	171.39c	26.83 a	23.50 a
10	4592.20b	1737.85ab	0.392b	0.114b	0.792a	0.76a	175.67b	190.42b	20.39 b	13.00 c
18	2054.40c	1524.03c	0.329b	0.096b	0.795a	0.76a	165.67c	193.75a	26.50 a	13.33b
<b>Fertilizer</b>										
Nano-ZnO	4163.30a	2069.81b	0.447a	0.120c	0.795ab	0.756a	219.71a	161.59d	32.00 a	9.33 d
Zn-Chelate	4365.10a	2365.46a	0.383b	0.126bc	0.794ab	0.754a	129.11d	173.33c	25.56 b	14.89 c
Mix	4209.80a	2259.72ab	0.397ab	0.140b	0.801a	0.771a	178.07c	179.93b	18.44 d	23.33 a
Check	4132.40a	1818.52c	0.407ab	0.175a	0.790b	0.754a	201.33b	226.48a	22.30 c	18.89 b
<b>Genotype</b>										
Morocco	3843.59b	1381.94c	0.415a	0.124b	0.795ab	0.751b	175.72b	196.78a	25.17 a	14.67 b
Nosrat	4402.67a	2232.57b	0.406a	0.133b	0.789b	0.765a	168.06c	197.58a	22.50 b	17.83 a
Khatam	4406.68a	2770.63a	0.406a	0.164a	0.801a	0.761ab	202.39a	161.19b	26.06a	17.33 a

Means within each column with similar letters are not significantly different (Duncan's 5%).

Due to significant differences in climatic conditions in the two growing seasons, and according to Bartlett's test, combined ANOVA was not advisable. Therefore ANOVA was carried out for each year separately.

### 3.1. Grain Yield

The effects of irrigation water quality (salinity) and genotypes on grain yield were highly significant in both years. The effects of fertilizer application on grain yield

were not significant in the first year (2012-13), but they were significant in the second year (2013–14).

The results of this study showed that salinity decreased grain yield. These adverse effects can be significantly reduced by Zn foliar application. The highest grain yield was produced in the Zn-chelate application treatments. Khatam had the highest grain yield due to a higher grain number per spike (Table 2). Consistent with these results, many researchers showed that the growth of plants

declined under saline conditions, but its degree depended on the level of salt, environmental conditions, type of plant, and stage of growth of barley (Shafaqat et al., 2012) and wheat (Pessarakli 1995).

### 3.2. Photosynthetic parameters

There are a lot of reports on photosynthetic characteristics under salt stress and, generally, photosynthesis is inhibited by salt stress (Venkateswarlu et al., 2012; Qiu et al., 2003; Munns, 2005; Chaves et al., 2009).

#### 3.2.1. Stomatal conductance (gs)

The results showed that salinity stress reduced stomatal conductance (gs). The effects of irrigation water quality (salinity) and genotype on gs were highly significant in both years. The effect of fertilizer on gs was not significant in the first year (2012-13), but it was significant in the second year (2013-14) (Table 2). The highest gs was obtained in the lowest salinity treatment (W1=2 dS/m) and the Nano-ZnO fertilizer application treatment in the first year and also in the check fertilizer application treatment in the second year. However, the Khatam genotype had the lowest gs in Nano-ZnO fertilizer application in the second year.

In addition, James et al. (2002) reported that salinity decreased by more than half in tolerant barley and wheat varieties, indicating that the sensitivity of the root system to NaCl could also be detected in leaves (Katsuhara et al., 2011).

#### 3.2.2. Chlorophyll fluorescence (Fv/Fm)

The effects of irrigation water quality (salinity) and fertilizer on Fv/Fm were not significant in both years, but the effect of genotype was significant. Salt-tolerant genotype (Khatam) had a higher maximum quantum efficiency (Fv/Fm) of PSII than a salt-sensitive genotype (Morocco) (Table 2). According to James et al. (2002), the Fv/Fm ratio of a salt-tolerant wheat genotype remained unchanged after salt treatment, indicating that there was no salt-induced decrease in intrinsic or actual quantum efficiency of PSII. In contrast, sensitive wheat genotypes showed a small but significant decline in Fv/Fm after salt

treatment. Also, unstressed plants had higher Fv/Fm values than salt-treated plants (James et al., 2008). This may be due to irreversible photoinhibition resulting from a sustained, high PPFD over the course of the experiment (Bilger et al., 1995).

### 3.2.3. Shoot nutrient element contents

The effects of irrigation water quality (salinity), fertilizer and genotype on shoot nutrient element content ( $K^+$ ,  $Na^+$ ,  $K^+/Na^+$ ,  $Fe^{2+}$ ,  $Zn^{2+}$  and  $Na^+Ca^{2+}$ ) were significant in both years.

#### 3.2.3.1. Potassium ( $K^+$ ) and $K^+/Na^+$ ratio

There was the highest  $K^+$  and  $K^+/Na^+$  ratio in the lowest salinity treatment. (W1=2 dS/m), Zn-chelate fertilizer application, and Khatam (salt-tolerant) genotype (Table 3). Salinity reduces  $K^+$  and  $K^+/Na^+$  content in shoots.  $K^+$  and  $K^+/Na^+$  contents in the shoots increased in Zn-chelate (foliar) application treatments and decreased in Nano-ZnO application treatments in comparison with the check (fertilizer without Zn).

The results of this study revealed that uptake of  $K^+$  and  $K^+/Na^+$  content was greater in tolerant and semi-tolerant genotypes compared with the sensitive genotype. Under saline conditions, a low  $K^+/Na^+$  ratio may indicate that Na impaired  $Ca^{2+}$ ,  $K^+$ , and Mg transport, which could disrupt plant metabolism and reduce plant growth.

Therefore, investigations dealing with the development of salt-tolerant varieties have concentrated on the uptake, transport, and accumulation of  $K^+$ ,  $Na^{2+}$ , and  $Ca^{2+}$  in plants (Morshedi and Farahbakhsh, 2012; Munns, 2005; James et al., 2008).

The concentrations of these nutrients and their ratios (e.g.,  $K^+/Na^+$  and  $Ca^{2+}/Na$ ) are reliable, useful, and widely used as screening parameters in ranking varieties for their tolerance to salt toxicity. Torabi (2010) confirmed the increasing  $K^+/Na^+$  ratio under salt stress. Tavakoli et al. (2010) reported that salt tolerant barley genotype "Afzal" produced higher dry mass compared to salt sensitive genotype under salt stress conditions (200 mM NaCl) and that higher tolerance in genotype Afzal was associated with a higher  $K^+/Na^+$  ratio in the shoots.

Table 3. Effects of water quality, fertilizer and genotypes on shoot nutrient contents (K, Na, K/Na and Na+Ca)

Treatments	K		Na		K/Na		Na+Ca	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
<b>Water quality (dS/m)</b>								
2	1.57 a	1.73 a	0.61 b	0.94 c	2.64 a	1.87 a	0.76 b	1.18 b
10	1.54 b	1.67 b	0.63 b	0.96 b	2.54 b	1.84 a	0.77 b	1.17 b
18	1.47 c	1.55 c	0.70 a	1.08 a	2.22 c	1.52 b	0.83 a	1.33 a
<b>Fertilizer</b>								
Nano-ZnO	1.49 c	1.48 d	0.63 b	0.97 b	2.44b	1.55 c	0.76 c	1.24 a
Zn-Chelate	1.57 a	1.83 a	0.65 a	0.94 c	2.53 a	2.02 a	0.82 a	1.24 a
Mix	1.52 b	1.57 c	0.66 a	0.97 b	2.39 b	1.69 b	0.80 ab	1.20 b
Check	1.54 b	1.73 b	0.64 a	1.08 a	2.38 b	1.71 b	0.79 b	1.24 a
<b>Genotype</b>								
Morocco	1.34 c	1.59 b	0.74 a	1.08 a	1.82 c	1.54 c	0.85 a	1.33 a
Nosrat	1.52 b	1.58 b	0.67 b	0.97 b	2.30 b	1.66 b	0.78 b	1.21 b
Khatam	1.73 a	1.79 a	0.55 c	0.93 c	3.18 a	2.03 a	0.74 c	1.14 c

Means within each column with similar letters are not significantly different (Duncan's 5%)

### 3.2.3.2. Sodium ( $\text{Na}^+$ ) and ( $\text{Na}^+ + \text{Ca}^{2+}$ )

The highest  $\text{Na}^+$  and  $\text{Na}^+ + \text{Ca}^{2+}$  rates in shoots were in the highest salinity ( $W3=18$  dS/m) and in check fertilizer application treatments, as well as in the Morocco genotype. Increasing salt stress causes an imbalance of the nutrient elements due to competition in uptake and toxicity in plants under salt stress (Pessaraki, 1999).

With increasing salinity, the ion concentration of  $\text{Na}^+$  and  $\text{Na}^+ + \text{Ca}^{2+}$  increased in the shoot tissues. This is confirmed by many authors (Khorshidi et al., 2009). There are some reports of antagonism between the absorption of  $\text{K}^+$  and  $\text{Na}^+$  at the root surface (Ahmadi et al., 2009).

### 3.2.3.3. $\text{Fe}^{2+}$ and $\text{Zn}^{2+}$

The highest  $\text{Fe}^{2+}$  content was in the lowest and highest salinity treatments in the first and second years, respectively (Table 2). The highest  $\text{Fe}^{2+}$  content was in Nano-ZnO fertilizer application and in check fertilizer application in the first and second year, respectively.

The Khatam genotype in the first year and the Morocco and Nosrat genotypes in the second year had the highest Fe content. The highest rate of  $\text{Zn}^{2+}$  was in the lowest salinity treatment ( $W1=2$  dS/m), and in Nano-ZnO fertilizer application treatments in the first year and in fertilizer mixtures in the second year, and for the Khatam genotype. Salinity decreased the total amount of  $\text{Zn}^{2+}$  in the shoots (about 36% in the second year) and Zn-chelate fertilizer application had the highest grain yield.

However, Khoshgoftar et al. (2004), Pahlavan-Rad and Pessaraki (2009) reported that salinity in irrigation water had no effect on  $\text{Zn}^{2+}$  concentration in the shoots of wheat. Shoot concentrations of  $\text{Zn}^{2+}$  varied among barley genotypes.

This may indicate that barley genotypes were different in their ability to accumulate  $\text{Zn}^{2+}$  both with and without  $\text{Zn}^{2+}$  fertilization. In the second year, the Khatam and Nosrat genotypes accumulated much more  $\text{Zn}^{2+}$  in their shoots than Morocco.

## 4. Discussion

The effects of irrigation water quality (salinity) on grain yield, stomatal conductance (gs) and shoot nutrient element content were highly significant in both years. With increasing salinity, grain yield, stomatal conductance,  $\text{K}^+$  ion, and  $\text{K}^+/\text{Na}^+$  ratio decreased, but  $\text{Na}^+$  ion and  $\text{Ca}^{2+} + \text{Na}^+$  ions increased. The effects of fertilizer application on grain yield, gs (only in the second year) and shoot nutrient element content were significant. Application of Zn-chelate fertilizer provided the highest grain yield,  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$  ratio and  $\text{Ca}^{2+} + \text{Na}^+$ . The Nano-ZnO fertilizer had the lowest  $\text{K}^+$  ion.

The effects of genotypes on grain yield, gs, Fv/Fm, and shoot nutrient element content were highly significant in both years. Sodium content in shoots increased due to salinity in all barley genotypes. However, the Morocco genotype maintained the highest leaf  $\text{Na}^+$  concentration. The Khatam genotype had the highest grain yield, gs, Fv/Fm,  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$ , and  $\text{Zn}^{2+}$  contents, and higher maximum quantum efficiency (Fv/Fm) than PSII. Based on the results of this

report, it can be concluded that Khatam and Nosrat genotypes maintained a lower level of  $\text{Na}^+$  in their shoots, and hence these genotypes can be considered as saline tolerant genotypes. Genotype Khatam (salt-tolerant) is comparatively higher in gs, Fv/Fm,  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$ , and  $\text{Zn}^{2+}$  content than Morocco (sensitive ones). The results showed that the tolerant genotype had fewer  $\text{Na}^+$  and  $\text{Ca}^{2+} + \text{Na}^+$  contents insensitive ones compared to sensitive ones. Moreover, all barley genotypes showed a decreasing trend in  $\text{K}^+$  content and the  $\text{K}^+/\text{Na}^+$  ratio due to salinity stress.

From the results of this study, it can be concluded that agrophysiological characteristics such as gs and leaf  $\text{K}^+/\text{Na}^+$  ratio may be used as potential traits for selecting barley genotypes with superior performance under salinity conditions. Furthermore, the genotypes that maintained a higher  $\text{K}^+/\text{Na}^+$  ratio can be considered salt tolerant.

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## Evaluation of five data mining algorithms in predicting monthly potential evapotranspiration (case study: Shiraz)

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### ABSTRACT

Data mining algorithms were used in this study to predict Shiraz's monthly potential evapotranspiration. The CART (Classification and Regression Trees), M5P, K-star, M5Rules, and REP-Tree (Reduced Error Pruning Tree) algorithms were used to predict potential evapotranspiration. Meteorological data from the Shiraz weather station from 2001 to 2016 were used in this study. The CART algorithm performed better in estimating monthly averages, according to statistical indicators. The maximum amount of potential evapotranspiration was reached when the sunshine hours exceeded 9.5 hours and the wind speed exceeded 0.3 meters per second, according to the results. When there was less than 9.5 hours of sunshine and the air temperature was less than 2 °C, the potential evapotranspiration rate was the lowest. The sensitivity analysis revealed that the parameters of sunshine hours, air temperature, wind speed, and relative humidity had a positive effect on the CART algorithm's performance in estimating monthly evapotranspiration.

## 1. Introduction

The actual evapotranspiration of the crop is considered equal to the water requirement of the crop, which is necessary to estimate in all irrigation projects. For this purpose, several computational methods have been proposed to estimate the potential evapotranspiration of grass as a reference crop in different parts of the world. After calculating the potential evapotranspiration using the crop coefficient, the reference crop evapotranspiration ( $ET_0$ ) is calculated and considered as the water requirement of the plant (Fooladvand, 2010).

Samadianfard and Panahi (2018) estimated the reference crop evapotranspiration of Tabriz city by both Thornthwaite and Hargreaves and data mining methods, including Support Vector Regression (SVR) and M5 Tree models. They concluded that data mining methods have a better ability to estimate reference crop evapotranspiration. Sameti et al. (2013) estimated the reference crop evapotranspiration of Shiraz and Kermanshah cities by Penman-Monteith and Hargreaves-Samani and compared their results with that of the M5 Tree model. They

concluded that the M5 Tree model performs as well as the abovementioned methods.

Due to the occurrence of the phenomenon of climate change, the use of indicators such as precipitation and potential evapotranspiration is inevitable (Asadi Zarch et al., 2017). So far, many studies have been conducted to accurately estimate evapotranspiration, which has led to a variety of methods for estimating evapotranspiration (Martí et al., 2015). The Penman-Monteith method is a physical algorithm that incorporates aerodynamic and thermodynamic aspects (Asadi Zarch et al., 2017). In Northeast India, by comparing the methods of reference evapotranspiration and examining different equations, the results obtained from the algorithms based on solar radiation and air temperature are similar to the results of the FAO Penman-Monteith algorithm (Pandey et al., 2016).

Among all the methods, the FAO method introduced a kind of standard in the interpretation and use of various expressions, such as potential evapotranspiration and evapotranspiration of the reference crop. The FAO has suggested that the hypothetical reference surface should be considered as the reference surface, just like the vast

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surface of green grass with uniform height, active growth, complete shading of the ground and sufficient water for evapotranspiration (Allen et al., 1998). In the FAO approach, the surface characteristics that affect evapotranspiration are clearly quantified (Itenfisu et al., 2003). The rate of evapotranspiration from the reference surface, without water shortage, is called the evapotranspiration of the reference crop and is indicated by ETO (Allen et al., 1998).

Data mining is the process of selecting, identifying, and algorithmically processing large amounts of data. Another definition is the process of selecting, exploring, and algorithmically mining large amounts of data, to uncover hidden relationships and achieve results that are clearly beneficial to the database's owner (Giudici, 2003). Data mining algorithms can automatically develop these equations from information contained in the data set (Crows, 1999). Tree algorithms have been evaluated by Mirhashemi and Panahi (2014b) to predict potential evapotranspiration and air temperature. It has been found that the performance of tree algorithms in predicting potential evapotranspiration has been acceptable. The performance of the M5P algorithm for estimation of the potential evapotranspiration and air temperature at Rasht meteorological station is evaluated. The results showed that the M5P algorithm has a better performance in predicting potential evapotranspiration (Mirhashemi and Panahi, 2014a). In a study conducted by Mirhashemi and Panahi (2014b) at Arak Meteorological Station, the performance of the CART algorithm in predicting potential evapotranspiration and air temperature was evaluated.

Conducting research to determine the water requirements of different crops during the growing season is necessary in order to prevent water wastage with proper planning and also to use appropriate management methods for the future. One of these issues that can be applied in line with the mentioned goals for the future is the prediction of ETo for the future in order to enable better use of available water resources with proper planning. Application of data mining algorithms is a suitable tool for various predictions. It was found that the performance of the M5P algorithm in predicting potential evapotranspiration is better than that of the minimum and maximum air temperatures of Sari meteorological station (Mirhashemi and Panahi, 2014a). Therefore, data mining algorithms can predict ETo to make a good prediction for the required water resources in the future. The purpose of this study is to evaluate the performance of tree algorithms in predicting monthly potential evapotranspiration in Shiraz.

## 2. Materials and methods

### 2.1. Study area

Shiraz is located in the central part of Fars province, at an altitude of 1486 meters above mean sea level in the mountainous region of Zagros and has a temperate climate. The city is bounded on the west by Mount Drak, on the north by the mountains of Bamo, Sabzpooshan, Chehel Magham and Baba Koochi (from the Zagros Mountains).

### 2.2. Data used

Monthly meteorological data of forty-six-year period from 1960 to 2005 of the Shiraz synoptic weather station" were used as inputs data, which includes: mean air temperature, sunshine hours, dew point, relative humidity, mean wind speed and saturation vapor pressure deficit. Many scientists have studied the Penman-Monteith equation to estimate ETO (Allen et al., 1998).

The Penman-Monteith method was ranked as the best method for all climatic conditions. Application of the Penman-Monteith equation FAO-56 requires data on sunshine, Wind speed, Air temperature, vapor pressure, and relative humidity, but all these input variables are not readily available in every location.

In developed countries, all climatic variables encountered in application of the FAO - 56 Penman-Monteith method are collected and can be used when needed, but in developing countries this is not the case

To predict the potential evapotranspiration of the next month, the six abovementioned variables were used. That was considered on a monthly basis, month after month, as the input data and the monthly potential evapotranspiration the next month as the output data. CART, M5P, K-star, M5Rules, and REP-Tree were used in this study to predict "potential evapotranspiration of the next month," therefore 75% of the data were used as the prediction algorithm and 25% as the test algorithm.

## 3. Results

The potential evapotranspiration of each month was calculated based on the potential evapotranspiration of the preceding month.

Values of monthly potential evapotranspiration are estimated by using the five algorithms and then compared with monthly potential evapotranspiration calculated by the Penman-Monteith equation. For the selection of the best algorithm for calculation of the monthly potential evapotranspiration, three statistical indices consisting of correlation coefficient (R), root mean square error (RMSE), and mean absolute error (MAE) were used for comparing the values calculated by data mining and the Penman-Monteith equation. As can be seen in Table 1, the CART algorithm, with a correlation coefficient of 0.92, RMSE of 1.02, and MAE of 1.45, is the best algorithm for estimation of the monthly potential evapotranspiration.

**Table 1. Comparison of five algorithms of data mining with three statistical indices.**

Algorithms	R	MAE	RMSE
K-star	0.75	3.12	3.45
CART	0.92	1.45	1.02
M5Rules	0.78	2.95	3.06
REP-Tree	0.84	3.41	3.20
M5P	0.52	4.18	4.91

The diagram in Figure 1 shows the linear diagram of the algorithm in predicting potential evapotranspiration.

According to the tree diagram, it is divided into two main branches. The dividing factor is the two main branches of the sunshine hours. According to the diagram, the maximum amount of potential evapotranspiration was reached when the sunshine hours were more than 9.5 hours and the wind speed was more than 0.3 meters per second. Increasing sunshine hours and wind speeds have a significant effect on increasing potential evapotranspiration. The lowest potential evapotranspiration rate was when the sunshine was less than 9.5 hours and the air temperature was less than 2 ° C. Air temperatures of less than 2 ° C have a great effect on reducing potential evapotranspiration. The prediction of this branch probably occurred in the autumn and winter seasons.

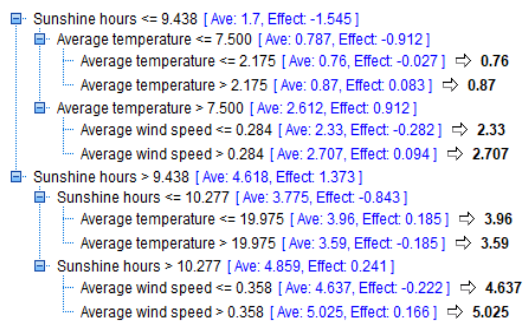


Figure 1. Linear diagram of the algorithm in predicting potential evapotranspiration

Figure 2 shows the importance of meteorological factors in predicting potential evapotranspiration by the CART algorithm. According to the figure, the factors of sunshine hours, air temperature, wind speed, and relative humidity have the greatest effect on predicting potential evapotranspiration.

### 3.1. Sensitivity analysis

To determine the most important factor for algorithming, "mean monthly potential evapotranspiration for the next month" via the CART algorithm was compared by changing the input data and using the statistical parameters. Which contains the "correlation coefficient", "root mean square error" and "mean absolute error" when compared to the third row, which includes five meteorological parameters, has the maximum "correlation coefficient" and minimum "square root error" and mean "absolute error".

As a result, five parameters were used to have the greatest impact on the performance of the CART tree algorithm.

The mentioned six parameters are listed in the first row, including the mean monthly air temperature, sunshine hours, dew point, relative humidity, wind speed, and saturation vapor pressure deficit, and the five parameters are listed in the last four rows, including sunshine hours, air temperature, wind speed, and relative humidity, which have the positive impact on the CART algorithm's proper functioning in estimating mean monthly evapotranspiration for the next month.

In Table 2, different combinations of input data were used to evaluate the sensitivity analysis of meteorological parameters.

In the first row, all meteorological parameters were used. In the next rows, the number of meteorological parameters decreased. Finally, according to the two statistical tests, the fifth row was selected as the best combination of input parameters. The fifth row of parameters, as in Figure 2, are sunshine hours, air temperature, wind speed and relative humidity.

These have a positive impact on the CART algorithm's proper performance in estimating average monthly evapotranspiration for the next month.

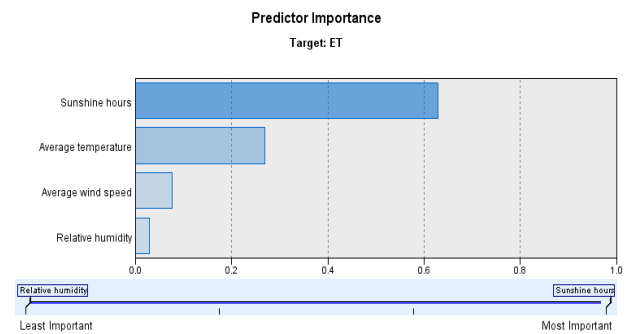


Figure 2. Importance of meteorological parameters in predicting potential evapotranspiration by CART algorithm.

The results of this study are similar to the results of Mirhashemi and Panahi (2014a) in predicting potential evapotranspiration and air temperature, in Rasht and Arak cities. They applied M5P and CART algorithms for the evaluation of the models. Also the results are in agreement with the results of Sameti et al. (2013) and Samadianfard and Panahi (2018).

It must be noted that in the current study, five data mining models were applied for evaluating different methods, while Sameti et al. (2013) have applied one model.

Table 2. The combination of input parameters to estimate the monthly potential evapotranspiration for the next month, using CART algorithm.

combination of input parameters*	R	MAE	RMSE
T, n, U, RH, T <sub>d</sub> , sd	0.92	1.45	1.02
T, n, U, RH, sd	0.92	1.50	1.89
T, n, RH, T <sub>d</sub> , sd	0.93	1.27	1.01
T, n, U, sd	0.91	2.20	2.89
T, n, U, RH	0.95	1.1	0.81
T, n, U	0.90	2.31	3.04
T, n	0.89	3.41	3.17
T, U	0.88	3.82	3.51
n, U	0.86	3.89	3.80

\*The parameters "dew point" (°C), "relative humidity" (percent), "sunshine hours" (h), "saturation vapor pressure deficit" (hPa.), "wind speed" (m/s), "mean monthly air temperature" (°C), are shown as T<sub>d</sub>, RH, n, sd, U, and T respectively.

### 4. Conclusions

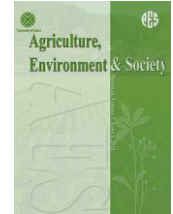
From this study, it can be concluded that the CART algorithm is a suitable model for estimation of the monthly potential evapotranspiration of the next month using meteorological elements. The CART algorithm can be used to estimate potential evapotranspiration in a variety of

stations that are deficient in recorded meteorological elements. Furthermore, in some areas where there is a shortage of meteorological data, using data mining algorithms is appropriate.

According to the results, by using the CART algorithm it was found that sunshine hours have the greatest effect on predicting potential evapotranspiration. After sunshine hours, air temperature, wind speed and relative humidity have the greatest effect on the prediction of potential evapotranspiration, respectively.

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## Effects of mycorrhiza symbiosis on seed yield and some physiological responses of chickpea genotypes

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### ABSTRACT

An experiment was conducted in 2014 in the research field of Ferdowsi University of Mashhad, Iran, to investigate the triplet symbiosis of chickpea, mycorrhiza, and rhizobium, as well as the responses of chickpea genotypes to these inoculations. The experimental design was a split-plot with three replications based on a randomized complete block design. The main plot included three mycorrhiza levels as a biological fertilizer (*Glomus mosseae*, *Piriformospora indica*, and non-used), while the subplot included nine chickpea genotypes. When compared to other treatments, *G. mosseae* significantly increased seed yield and dry matter of chickpeas from mid-season onward. Arbuscular mycorrhiza significantly increased chlorophyll a and b levels, as well as carotenoids and SPAD levels. MCC537 outperformed the other genotypes in terms of seed yield and dry matter during the growing season and at harvest time. MCC537, MCC427, and MCC392 genotypes had the highest levels of carotenoids and SPAD readings. It appears that using *G. mosseae* in conjunction with rhizobium can improve the physiological traits and seed yield of chickpea.

### 1. Introduction

During the last few decades, the indiscriminate use of chemical fertilizers to achieve greater agricultural productivity has had adverse environmental consequences due to the increasing contamination of soil and water resources (Kranz et al., 2020). However, the idea of returning to nature and less use of chemical fertilizers and pesticides, and the incremental tendency for people to use organic products, have drawn more attention to the use of biological fertilizers. The use of biological resources instead of chemical resources has an important role in soil fertility and environmental protection (Zaidi et al., 2003). The use of bio-fertilizers is considered, including strategies to improve nutrient supply in sustainable agriculture. In other words, one of the main pillars of sustainable agriculture is the use of bio-fertilizers in agricultural ecosystems, with the approach of minimizing the use of chemical inputs (Sharma, 2002).

The importance of pulses in agricultural systems has long been known, but because of the environmental

problems that are created in conventional agricultural systems, their importance has been increased. In today's agriculture, nitrogen is one of the main limiting factors for agricultural production (Lucisk et al., 2002). It has been indicated that nitrogen has the highest effect on increasing leaf area index and crop growth rate among essential elements. Nitrogen deficiency often occurs at critical growth stages of the plant, with symptoms such as yellowing of the leaf tissue (called chlorosis) (Arshadi and Asgharipour, 2011). However, pulses, because of the symbiosis with rhizobium bacteria, are self-sufficient in terms of supplying required nitrogen as carbon supply and have a significant role in maintaining nitrogen balance in the world (Voisin et al., 2013). Chickpea (*Cicer arietinum* L.) is the second most important food legume after dry beans, which are cultivated on more than 13.5 million ha in the producer countries and produce more than 8 million tons with a high protein content (22–24%) (FAOSTAT, 2018; Gaur et al., 2014). About 3.2% of the world's chickpeas are produced in Iran, and in this country, chickpeas among pulses have been allocated the biggest

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<http://dx.doi.org/10.22034/aes.2021.144190>

area under cultivation, at 641000 ha (FAOSTAT, 2018). However, both the irrigated and rain-fed yields of chickpea are low compared to other producing countries of this crop.

One of the most effective factors for increasing the seed yield of chickpea is a symbiosis with mycorrhiza. Rhizobium colony formation in roots by mycorrhizal fungi can provide a suitable condition for nodulation of rhizobium, because they enhance the availability of phosphorus for nitrogenase enzymes involved in rhizobium bacteria (Diouf et al., 2003). Mycorrhizal fungi are divided into two categories: endo-mycorrhiza (AMF: Arbuscular Mycorrhizal Fungi) and ecto-mycorrhiza (Abbott and Murphy, 2007). AMFs generally belong to the Zygomycetes classes (that are newly named Glomeromycetes) and ecto-mycorrhiza are mainly Basidiomycetes (Abbott and Murphy, 2007). Recently, a new species of ecto-mycorrhiza has been identified with the name *Piriformospora indica* that acts as an AMF and is an endophyte fungus (Verma et al., 1998). That is why it is called "pseudomycorrhiza." Some recent research has shown that symbiosis with *P. indica* increases the tolerance

of crops to adverse environmental conditions (Baltruschant et al., 2008; Stein et al., 2008). Therefore, this study was performed to investigate the effects of different genotypes inoculated with rhizobium, AMF, and pseudo-endomycorrhiza on seed yield and some physiological characteristics.

## 2. Materials and methods

The experiment was carried out in 2014 at the Faculty of Agriculture, Ferdowsi University of Mashhad (Iran). For one year before conducting the experiment, the land was left fallow. The experimental design was a split-plot based on a randomized complete block design with three replications. Main plots consisted of three levels of mycorrhiza as a biological fertilizer (arbuscular mycorrhiza of *Glomus mosseae*, pseudo-endomycorrhiza of *Piriformospora indica*, and non-used mycorrhiza) and subplots consisted of nine genotypes of chickpea (high potential yield genotypes selected by the Institute of Plant Sciences, Ferdowsi University of Mashhad seed bank (Table 1).

Table 1. Traits of chickpea genotypes that were used in the experiment.

Identifying code in seed bank of Iant Institute	Origin and name of genotypes
MCC* 80	Iran - 5311
MCC 358	Iran – Karaj cv.
MCC 361	Iran – Jam cv.
MCC 392	Iran – native lats of Kermanshah
MCC 427	Iran – native lats of Bojnurd
MCC 537	Iran – native lats of Gonabad
MCC 693	Iran
MCC 696	Iran
MCC 950	Iran – Hashem cv.

\* Mashhad Chickpea Collection

Before planting, soil samples were taken to a depth of 0.3 m and characteristics including pH, organic matter, EC, soil texture, macro (N, P, K) and micro (Fe, Cu, Mn, Zn) elements were determined (Table 2). The soil texture of the experimental site was silt loam. Based on laboratory fertilizer recommendations, the phosphorus and potassium content of the soil was enough, so there was no need to apply phosphorus and potassium fertilizer. However, at the time of planting 40 kg/ha of urea as a starter and, a month before planting, 20000 kg/ha of manure were applied to improve soil organic carbon. After land preparation (including plow, disk, leveling, and furrower handling), plants were sown on March 11, 2014. The distance between and within rows was 0.1 and 0.5 m, respectively, and five rows were considered in each plot, with two rows set aside as a margin. The width of the plots was 7.5 m, and in each

row, 75 seeds were planted by hand at a depth of 0.05 m. Seeds were disinfected with a 5% sodium hypochlorite solution. In order to infect the seeds with the symbiotic rhizobium bacteria of chickpea-*Mesorhizobium ciceri*, the bacterial inoculum was spread with seeds and uniformly used for all treatments.

In order to infect the soil with *G. mosseae*, in the treatment involved, 30 g of soil infected with fungal mycelium was poured onto the seed placement. To infect the soil with *P. indica*, after developing roots from 2-day-old germinating, seeds of chickpea were immersed in the source of the *P. indica* and then planted (Harrach, 2009). To apply *P. indica*, first its culture medium as a solid medium was prepared (Kumar et al., 2011). Then *P. indica* was inoculated on Petri dishes, and the plates were moved into incubators to grow and propagate.

Table 2. Characters of studied soil.

pH	EC (dS/m)	OC (%)	N (ppm)	P (ppm)	K (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
7.29	4.26	0.87	861	38	413	7.62	1.52	1.41	17.15

Weeding was performed three times on May 7, 13, and 25 during the growing season. On May 14 and 20, diazinon concentrate was sprayed twice to control the Heliothis

worm. For all treatments, irrigation was applied after planting, beginning of flowering, and beginning of pod forming (when the three plants of the three central rows of

each plot had gone to flower, it meant flowering, and when three plants of the three central rows of each plot had gone to pod, it meant pod forming (IBPGR, 1993)).

The amounts of a, b, and total chlorophyll and carotenoids of leaves were determined by using a spectrophotometer after chlorophyll extraction with 80% acetone at 50% flowering, from a developed leaf at the top of the canopy (Wellbum, 1994; Nikolaeva et al., 2010). The results were expressed as 1 g of leaf fresh weight.

In the mid-flowering, the SPAD chlorophyll content of leaves was measured by monitoring three plants per plot. The tip of the leaflet of the first leaf, fully developed on the upper stem, was used for the chlorophyll meter. Measurements were made at a central spot on the leaflet, between the midrib and the leaf margin (Wu et al., 2006; Pepó and Vári, 2016). During the growing season at 32, 51, 65, 75, 81, and 87 days after planting, three plants were randomly selected from the upper half of each plot, and then the dry matter was assessed by placing them in an oven at 75°C for 48 h. Plants were harvested on the bottom half of each plot allocated for the yield evaluation, and grain yield was measured. It is noteworthy that the MCC80 genotype was ready to be harvested on June 21, 2014. On June 27, 2014, the MCC696, MCC693, MCC950, MCC358, MCC361, MCC392 and MCC427 genotypes were ready to be harvested, and the MCC537 genotype was ready to be harvested on June 29. Data analysis was performed by using M-STAT-C software, and mean comparisons were performed by using the Duncan test. Treatments were compared at the probable level that was significant as 1 or 5%.

### 3. Results and discussion

#### 3.1. Dry Matter

At first sampling, biological fertilizer and chickpea genotypes did not significantly affect the dry matter (DM) of chickpeas. However, at the second (51 DAS–Day After Sowing), third (65 DAS), fourth (75 DAS), and fifth (81 DAS) samplings, the interaction of biological fertilizer and chickpea genotypes was significant ( $P \leq 0.01$ ). The simple effects of biological fertilizer and chickpea genotypes were also significant at the sixth (87 DAS) sampling (Table 3). In these samplings, the DM of those exposed to rhizobium and mycorrhizal treatments was significantly higher than that of other plants. During the growing season, the most DM was considered in treatments of MCC537 and MCC427 + rhizobium with mycorrhiza (Table 5). On the other hand, since the second sampling (51 DAS) towards the end of the growing season, the DM of MCC537 and MCC427 genotypes were treated with rhizobium, and mycorrhiza was higher than the other genotypes (Table 5, Figure 1). It seems that these two genotypes have been more successful in communicating symbiosis with rhizobium and mycorrhiza than other genotypes. In the sixth sampling, there was no significant difference between treatments of rhizobium (alone) and rhizobium with pseudo-endomycorrhiza, but DM was significantly higher in the integrated application of rhizobium and mycorrhiza. Also, in the last sampling, the DM of MCC537 and MCC427 was significantly higher than the other genotypes, and MCC80 showed the lowest DM among all nine study genotypes. It seems that MCC537 and MCC427 genotypes were ranked first and second for DM production.

Table 3. ANOVA for DM.

S.O.V	df	1 <sup>st</sup> sampling of DM	2 <sup>nd</sup> sampling of DM	3 <sup>rd</sup> sampling of DM	4 <sup>th</sup> sampling of DM	5 <sup>th</sup> sampling of DM	6 <sup>th</sup> sampling of DM
Replication	2	0.120	0.436	11.43	147.4	34.2	485.7
Bio-fertilizer (A)	2	0.030 <sup>ns</sup>	37.79 <sup>**</sup>	3596 <sup>**</sup>	8233 <sup>*</sup>	15387 <sup>*</sup>	15912 <sup>*</sup>
Error (a)	4	0.459	0.229	5.74	942.3	1016	1065
Genotype (B)	8	0.011 <sup>ns</sup>	38.30 <sup>**</sup>	4570 <sup>**</sup>	10420 <sup>**</sup>	16153 <sup>**</sup>	20873 <sup>**</sup>
A * B	16	0.017 <sup>ns</sup>	2.26 <sup>**</sup>	363.2 <sup>**</sup>	1082 <sup>**</sup>	2211 <sup>**</sup>	1811 <sup>ns</sup>
Error (b)	48	0.172	0.818	10.55	321.5	699.4	1200
Total	80	-	-	-	-	-	-
C.V. (%)	-	16.19	6.30	2.22	7.03	7.31	9.93

ns: non-significant      \*: significant in 5% level      \*\*: significant in 1% level

Table 4. ANOVA for traits of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, SPAD readings and seed yield.

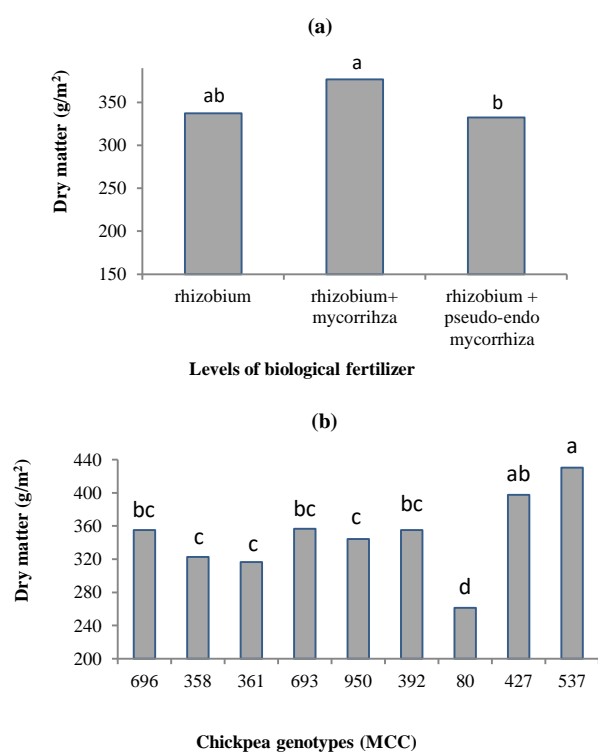
S.O.V	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	SPAD readings	Seed yield
Replication	2	0.0005	0.004	0.0002	0.001	0.197	9236
Bio-fertilizer (A)	2	0.012 <sup>**</sup>	0.007 <sup>*</sup>	0.021 <sup>ns</sup>	0.088 <sup>*</sup>	78.70 <sup>**</sup>	602275 <sup>**</sup>
Error (a)	4	0.001	0.001	0.004	0.010	1.495	11537
Genotype (B)	8	0.003 <sup>**</sup>	0.010 <sup>**</sup>	0.150 <sup>**</sup>	0.178 <sup>**</sup>	162.4 <sup>**</sup>	2313273 <sup>**</sup>
A * B	16	0.0003 <sup>ns</sup>	0.003 <sup>*</sup>	0.003 <sup>ns</sup>	0.024 <sup>ns</sup>	6.70 <sup>ns</sup>	72262 <sup>**</sup>
Error (b)	48	0.0004	0.001	0.002	0.018	12.01	15762
Total	80	-	-	-	-	-	-
C.V. (%)	-	7.04	12.66	7.56	6.46	12.72	8.29

ns: not-significant      \*: significant in 5% level      \*\*: significant in 1% level

#### 3.2. Chlorophyll Content

The effect of biological fertilizer on the chlorophyll a of chickpea leaves was significant at a 1% probability level (Table 4). Chlorophyll a of rhizobium and mycorrhizal treatments was significantly higher than the other

treatments, and in comparison with treatments of rhizobium (alone) and rhizobium with pseudo-endomycorrhiza, they increased chlorophyll a by about 11.53% and 12.30%, respectively. There was no significant difference between using rhizobium alone and rhizobium



Means with a common letter have not significantly different together based on Duncan's test at 5% for biological fertilizer and at 1% for chickpea genotypes.

**Figure 1. Effect of biological fertilizer (a) and chickpea genotypes (b) on 6<sup>th</sup> sampling of DM.**

**Table 5. Mean comparisons for interactions of biological fertilizer and chickpea genotypes on DM, chlorophyll b and seed yield.**

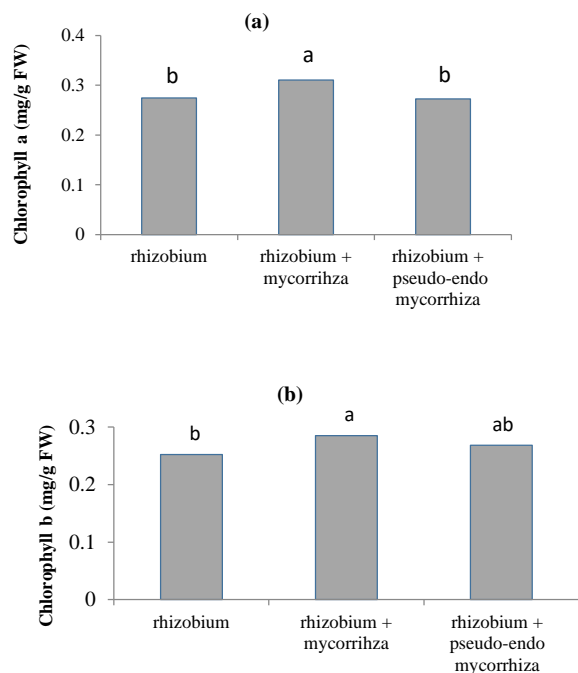
Biological fertilizer	(MCC) genotype	2 <sup>nd</sup> sampling of DM (g/m <sup>2</sup> )	3 <sup>rd</sup> sampling of DM (g/m <sup>2</sup> )	4 <sup>th</sup> sampling of DM (g/m <sup>2</sup> )	5 <sup>th</sup> sampling of DM (g/m <sup>2</sup> )	Chlorophyll b (mg/g FW)	Seed yield (kg/ha)
Rhizobium	696	14.80 c-g	141.9 gh	240.2 e-h	348.4 b-d	0.243 c-f	1134 i-k
	358	11.51 jk	118.1 mn	226.1 f-h	330.6 c-f	0.256 a-f	925 kl
	361	12.26 h-k	126.3 kl	230.8 f-h	325.7 d-f	0.213 fg	876 kl
	693	14.08 d-i	139.3 gh	246.1 d-g	349.3 b-d	0.150 g	1370 g-j
	950	13.36 e-j	137.0 hi	233.2 f-h	348.2 b-d	0.228 ef	790 l
	392	14.42 c-h	146.7 fg	249.2 d-g	360.0 b-d	0.283 a-f	1907 de
	80	13.33 e-j	124.4 k-m	208.0 gh	281.0 ef	0.291 a-f	1488 gh
	427	15.58 b-e	156.8 e	268.3 c-f	375.2 b-d	0.308 a-e	1957 c-e
	537	17.33 b	171.9 d	290.9 cd	396.3 bc	0.301 a-e	2085 b-e
Rhizobium+ Mycorrhiza	696	15.22 b-f	152.5 ef	244.6 e-h	368.5 b-d	0.235 d-f	1187 h-k
	358	11.93 i-k	127.0 kl	237.4 f-h	342.0 b-e	0.259 a-f	1285 h-j
	361	12.94 f-k	128.3 j-l	231.3 f-h	332.9 b-f	0.246 b-f	1185 h-k
	693	15.22 b-f	151.7 ef	265.6 d-e	377.2 b-d	0.329 a	1431 g-i
	950	15.21 b-f	153.9 ef	270.6 c-f	367.9 b-d	0.228 h-j	1124 i-k
	392	16.28 b-d	166.8 d	284.3 c-f	390.5 b-d	0.325 ab	2352 b
	80	14.39 c-h	131.3 i-k	223.5 f-h	324.2 d-f	0.315 a	1615 fg
	427	20.26 a	205.7 b	338.5 b	480.1 a	0.301 e	2226 bc
	537	19.44 a	217.7 a	381.2 a	520.8 a	0.326 b-d	2730 a
Rhizobium+ Pseudo-endo mycorrhiza	696	14.81 c-g	137.8 hi	247.3 d-g	355.1 b-d	0.241 h-j	1085 j-l
	358	10.69 k	140.6 gh	257.5 c-f	369.5 b-d	0.254 i-k	924 kl
	361	11.90 i-k	120.9 l-n	224.3 f-h	323.8 d-f	0.255 f-h	1094 j-l
	693	14.08 d-i	134.8 h-j	241.4 e-h	363.2 b-d	0.251 f	1375 g-j
	950	12.72 g-k	123.3 lm	239.1 e-h	353.7 b-d	0.232 f	1347 g-j
	392	12.40 h-k	138.6 hi	240.9 e-h	343.6 b-e	0.296 cd	1817 ef
	80	11.61 jk	113.7 n	198.8 h	273.1 f	0.280 de	1302 h-j
	427	15.18 b-f	158.3 e	269.0 c-f	369.4 b-d	0.303 a-c	2099 b-e
	537	16.67 bc	180.3 c	300.3 bc	400.6 b	0.302 a-c	2203 b-d

Means that have a common letter, have not significantly difference together based on Duncan's test at 1%.

with pseudo-endomycorrhiza (Figure 2a). The effect of biological fertilizer on chlorophyll b was significant at a 5% probability level (Table 4). The amount of chlorophyll b using rhizobium and mycorrhizal treatment was significantly higher than using rhizobium alone (Figure 2b). However, the effect of biological fertilizer on the total chlorophyll of the leaf was not significant (Table 4). The effect of chickpea genotypes on chlorophyll a of the leaf was significant ( $P < 0.01$ ) (Table 4). MCC537 showed the highest chlorophyll content of 0.3101 mg/g, but no significant difference was found between genotypes of MCC427, MCC392, MCC696, and MCC693 with MCC537. There were no significant differences between MCC361, MCC358, MCC80, and MCC950 (Figure 3a).

For chlorophyll b, genotypes of MCC427, MCC392, MCC80, and MCC537 were in the same significant class, and genotypes of MCC358, MCC361, MCC696, MCC693, and MCC950 were in the other significant class (Figure 3b). Moreover, the effect of chickpea genotypes on the total chlorophyll of chickpea leaves was significant at the 1% probability level (Table 4). The highest total chlorophyll was obtained for MCC537 and the lowest for MCC950. However, between genotypes of MCC537, MCC427, MCC392, MCC80, and MCC693 and also between genotypes of MCC950, MCC358, MCC361, and MCC696, there was no significant difference (Figure 3c).

The effect of chlorophyll a as a major factor in the center of photosynthetic reaction, the role of chlorophyll b as an antenna pigment, and the phenomenon of energy funnel have long been known (Taiz and Zeiger, 2006), and increasing their amounts by using a combination of rhizobium and mycorrhizal will affect the photosynthesis rate and thus crop photosynthetic capacity. As regards the three study genotypes of MCC537, MCC427, and MCC392, they accounted for the maximum amounts of three types of chlorophyll a, b, and total. It seems that these three genotypes have a high potential for the production of chlorophyll.

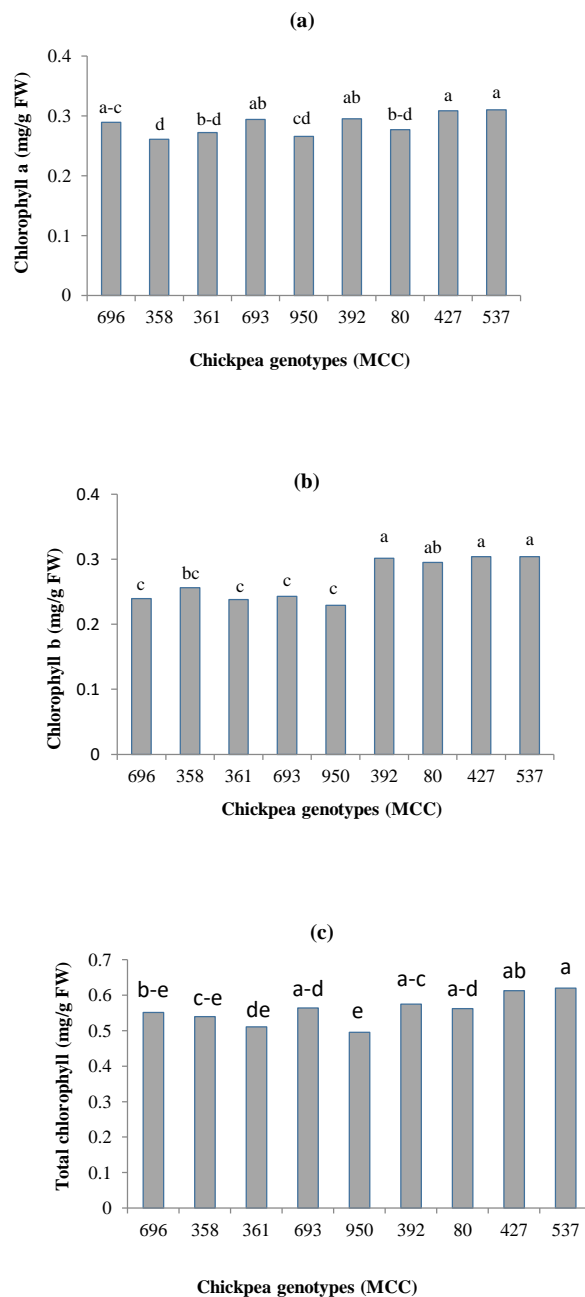


Means with a common letter have not significantly different together based on Duncan's test at 5% for chlorophyll b and at 1% for chlorophyll a.

**Figure 2. Effect of biological fertilizer on contents of chickpea chlorophyll. (a) and (b) are effects of biological fertilizer on chlorophyll a and chlorophyll b, respectively.**

### 3.3. SPAD Readings

The effect of biological fertilizer on the SPAD readings of chickpea leaf was significant at the 1% level (Table 4). So, SPAD readings of rhizobium and mycorrhizal treatments were significantly higher than the other treatments, and this treatment, in comparison with treatments of rhizobium (alone) and rhizobium with like-endo mycorrhiza, increased SPAD readings by an amount of 8.03% and 11.39%, respectively (Figure 4). According to the superiority of the characteristics of rhizobium and mycorrhizal treatment in tissue nitrogen of plants and chlorophyll (especially chlorophyll a), it seems reasonable to the superiority of SPAD readings of this treatment compared to other treatments. Because nitrogen is an integral part of the chlorophyll structure, and the chlorophyll meter SPAD-502 shows an estimate of the



**Figure 3. Effect of chickpea genotypes on contents of chickpea chlorophyll. (a), (b) and (c) are effects of chickpea genotypes on the chlorophyll a, chlorophyll b and total chlorophyll, respectively.**

amount of nitrogen that is in leaf chlorophyll content.

The effect of chickpea genotypes on the SPAD readings of chickpea leaves was significant at the 1% level (Table 4). The MCC537 genotype, with 33.84%, showed the highest SPAD readings. Of course, this genotype was in the same significant group as MCC427 and MCC392. On the other hand, the MCC358 and MCC361 genotypes compared with other genotypes in this study showed the lowest SPAD readings (Figure 5). Given that the chlorophyll meter SPAD-502 is an estimate of the amount of leaf chlorophyll (Martinez and Guiamet, 2004). It seems that some chickpea genotypes have a higher ability to synthesize chlorophyll in their leaves.

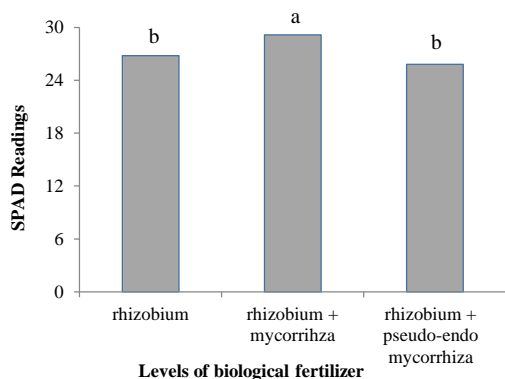


Figure 4. Effect of biological fertilizer on SPAD readings of chickpea carotenoids.

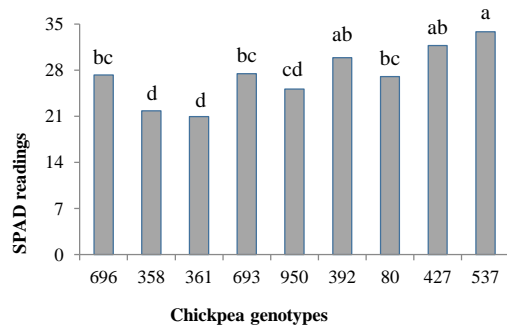


Figure 5. Effect of chickpea genotypes on SPAD readings of chickpea carotenoids.

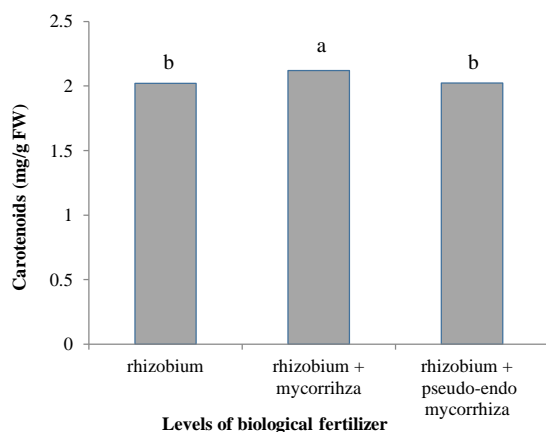


Figure 6. Effect of biological fertilizer on contents of chickpea carotenoids.

### 3.4. Carotenoids

The Effect of biological fertilizer on the carotenoids of leaves was significant at a 5% probability level (Table 4). Carotenoids of rhizobium and mycorrhizal treatments were significantly higher than the other treatments, and this treatment, in comparison with treatments of rhizobium (alone) and rhizobium with pseudo-endomycorrhiza, increased carotenoids by 4.71% and 4.62%, respectively. Indeed, there was no significant difference between treatments of rhizobium alone and rhizobium with pseudo-endomycorrhiza (Figure 6). At the 1% probability level, the

effect of chickpea genotypes on leaf carotenoids was significant (Table 4).MCC537 had the highest carotenoid content, while MCC950 had the lowest. Indeed, between the genotypes of MCC537, MCC427, and MCC392, there was no significant difference. Also, there were no significant differences between MCC950, MCC358, MCC361, and MCC696 (Figure 7).

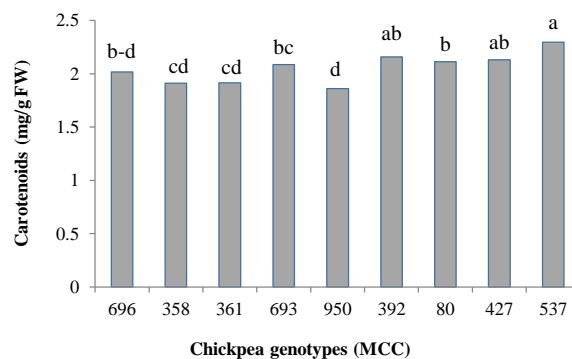


Figure 7. Effect of chickpea genotypes on contents of chickpea carotenoids.

Carotenoids are a large group of isoprene molecules that are made by all photosynthetic tissues and some non-photosynthetic organs. Carotenoids are divided into hydrocarbon carotenes, such as lycopene and beta-carotin or G-xanthophyll (Andrew et al., 2008). It seems that the combined use of rhizobium and mycorrhiza in providing substrate for the biosynthesis of carotenoids has been more successful than the other two treatments. Considering that the three genotypes of MCC537, MCC427, and MCC392 accounted for the maximum amount of carotenoids, it seems that the three genotypes have a high potential for the production of carotenoids. These results indicate that the chickpea genotypes have different capabilities in the production of photosynthetic pigments. In another study, the physiological responses of 35 chickpea genotypes were examined under drought stress and non-stress conditions. Carotenoids content of genotypes Flip03-63C, Flip03-87C, Flip05-59C, Flip05-153C, Flip05-74C, and Flip05-143C decreased less under drought stress than other genotypes in this study (Talebi et al., 2013).

### 3.5. Seed Yield

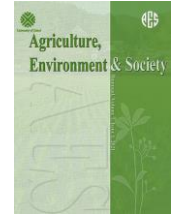
The interaction effect of biological fertilizer and chickpea genotypes on the seed yield of chickpea was significant at a 1% probability level (Table 4). Seed yield in the treatment of MCC537 + rhizobium with mycorrhiza was significantly higher than in other treatments (Table 5). Seed yield differences between genotypes studied in this research were ascribed to different capabilities of production potential in these genotypes. In other words, there was a considerable difference between chickpea genotypes in terms of yield potential. In general, almost all genotypes' seed yields were higher in treatments of integrated application of rhizobium and mycorrhiza than in treatments of rhizobium (alone) and rhizobium with pseudo-endomycorrhiza. These findings reflect the success of chickpea, mycorrhiza, and rhizobium triplet symbioses,

as well as the better role of mycorrhiza in providing nutrients required for plant growth and the better distribution of photosynthesis products between chickpea photosynthetic organs and sinks compared to the other treatments. Zaidi et al. (2003) reported that the use of rhizobium along with the phosphate-solubilizing microorganisms and AMF causes an increase in rhizobium nodule dry weight, and that this function can enhance the seed yield of chickpea. It seems that rhizobium with mycorrhiza treatment, rather than the other two treatments, has been more successful in the absorption of water and nutrients for chickpea. This phenomenon increases the photosynthetic capacity of the plant and leads to a better allocation of photosynthetic products to the seeds of chickpea. Solaiman et al. (2005) concluded that the use of rhizobium along with arbuscular mycorrhiza for chickpea could cause an increase in seed yield because of increasing nodule dry weight in comparison to the application of rhizobium alone.

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## An examination of the impact of agricultural crop insurance on crop production and gross margin of rural farms

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### ABSTRACT

Because of the agricultural sector's importance in the national economy, and because it accounts for 17 percent of GDP and 22 percent of job labor, it is critical to support it as much as possible. Agricultural product insurance is one of the most important governmental support levers in the agricultural sector. It not only compensates farmers for the losses caused by the small savings of a large number of farmers, but it also provides more security for agricultural producers, lowering production risks significantly. So, in this study, we attempted to use positive mathematical programming (PMP) to examine the effects of introducing wheat insurance on wheat cultivation and farmer gross margins in the Sistan region. These farmers were divided into three groups based on the size of their cultivated farms: (1) small group (less than 3 hectares), (2) average-sized group (4-10 hectares), and (3) large group (more than 10 hectares). The results showed that after introducing wheat insurance, the farmer accepted it, and the cultivated area of wheat increased from 2 hectares to 2.01 hectares, while the farm's gross return increased from 18423290 Rials to 18511721 Rials, i.e., 0.479 percent more than those farmers who did not participate in the insurance scheme. On average-sized farms, implementing this insurance increased wheat cultivated area from 4.8 hectares to 4.858 hectares, and farm gross return increased from 48803550 Rials to 49291580 Rials, i.e., about 1% more than farmers who did not participate in the scheme. Following this plan, the cultivated area of wheat increased from 16 hectares to 16.445 hectares, and the farm's gross return increased from 139151140 Rials to 142421200 Rials, a 2.35 percent increase over those who did not participate in the scheme.

### 1. Introduction

In most developing countries, agriculture has gained special importance. Since it includes various activities for providing a part of the national income in foreign currency and food security, it has a remarkable role in developing the economic-social growth of these countries. On the other hand, increased reliance on natural and environmental factors, as well as market volatility in agricultural activities, pose their own risks. Hence, the survival and durability of the agricultural sector require the serious support of its own producers and investors. Meanwhile, numerous risks, such as production, price, or financial market risks, arising from uncertainty about the agricultural policies and activities of the government, as well as human risks, affect the

income and welfare levels of these farmers (Shokri and Samadi, 2010). The presence of various risks makes farmers rethink their investments. One of the most important challenges for agricultural planners and policy-makers is to make deliberate and accurate decisions and policies to reduce the income fluctuations of agricultural producers, especially those producers of strategic products (Faraji and Mirdamadi, 2007). There are different approaches titled "the risk management tools," which can decrease the destructive effects of the risks involved in agricultural activities. Risk management is the hazardous management of implementing different methods, tools, and policies to lower the negative impacts of various risks (Tomek and Peterson, 2000).

Insurance of agricultural products is a supporting tool for controlling and managing risk and increasing the security margin of investment in the agricultural sector, which has attracted the attention of policymakers, especially during recent decades. Agricultural insurance is

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a unique financial solution that protects the farmers against possible damage and low income, so it can be considered as an accurate and precise source for the farmers to accept innovations and revisions in their production methods (Forohideh and Tarazkar, 2009).

### 1.1. The definition of the problem

Agriculture is always accompanied by risk. The presence of risk and the producers' reaction to it affect the form and content of policies made by the agricultural sector. In fact, variety, instability, and risk are three main factors that play a significant role in the motivation and programming of policy-making processes in this sector (Hosseini and Gholizadeh, 2008).

Instability in agricultural incomes and damages incurred to the producers that are the results of the uncontrollable above-mentioned risks had forced many developing countries to program for a more stable agricultural income. Among different economic sectors, agriculture is more subjected to risk. So, due to its numerous effects, insurance can lead to satisfactory rural development, either at a micro or macro level, and it can achieve simultaneous progress for all sectors (Vedenov and Power, 2008).

Insurance policies for agricultural products can achieve this goal by taking possible risks and eliminating the rest of these uncertainties, so that there will be a balance between the demand and supply of the insurance, and the insurance programs will be more efficient and financially, they will rely on their own (Shokri and Samadi, 2010).

### 1.2. Significance

One of the supporting levers of the government is the insurance of agricultural products. Using this strategy, it can collect the little savings of numerous farmers paid as insurance and use them as compensation for possible agricultural damage. On the other hand, it can increase agricultural producers' security and reduce production risks. Therefore, the insurance of agricultural products can be considered as one of the most essential and effective solutions (Hayati et al., 2010).

However insurance is still an unknown phenomenon for many farmers, mainly because of a low culture of using insurance. Most farmers have no information about the positive, supportive effects of using insurance, and they consider it a waste of money (Iravani et al. 2006). %80 of these farmers are illiterate and less educated, whereas this figure has risen to %85 in Sistan and Baluchistan. Furthermore, only 4.2% of Sistani farmers have a university degree, and only 0.6% have an agricultural degree.

Hence, in spite of various publicities about agricultural products, farmers are not so willing to use this supportive lever (Shokri and Samadi, 2010). In this study, we have used the positive mathematical method for modeling the Sistani farmers' Wheat insurance. The aim of the study was to examine the impact of wheat insurance on wheat farm management and gross margins in Sistan.

## 2. Methodology

The main idea in PMP is to use the information available in the dual variables of the calibration limits. These dual figures are used to clarify the nonlinear objective function that rebuilds the observed levels of activities into the optimal solution for the new programming problem with no calibration limit (Salami and Einollahi Ahmadabadi, 2001). The PMP has three stages:

Stage 1: calculating shadow prices with supplementary linear programming

Mathematically, it is possible to show the first stage of PMP with a simple linear programming model for maximizing the planned gross margin:

$$\begin{aligned} \text{Max } Z &= \text{GM}'X && \text{Eq. 1} \\ \text{s.t.} & && \\ AX &\leq b && [\pi] \\ X &\leq (X^0 + e) && [\lambda] \\ X &\geq 0 && \end{aligned}$$

Where  $Z$  is the objective function that must be maximized,  $X$  is the activities' vector,  $A$  is the matrix of technical coefficients, and  $b$  and  $\pi$  are the available resource vector and dual variables, respectively (or shadow prices). In these resources,  $e$  and  $\lambda$  are the vector of small positive numbers, and the dual variable of calibration limits,  $x^0$  is the activity level observed in the base year.  $\text{GM}$  is the product gross margin vector that for each activity is as follows:

$$\text{GM} = (\text{YP}) - C \quad \text{Eq. 2}$$

Where  $P$  is the cost of the crop,  $Y$  is the performance of the crop, and  $C$  is the total variable cost.

Stage 2: calculating the nonlinear calibrated cost function

In the second stage, the amount  $\lambda$  is used to calculate a nonlinear variable cost function. Sometimes, for simplicity and lack of strong reasons for choosing other variables, we use the following quadratic variable cost function:

$$C^V = d'x + \frac{1}{2}x'Qx \quad \text{Eq. 3}$$

Where  $C$  represents variable costs,  $d$  represents an axis ( $n \times 1$ ) of parameters related to the linear part of the cost function, and  $Q$  represents a symmetric positive definite matrix ( $n \times n$ ) of parameters related to the quadratic variable cost function. This nonlinear variable cost function is obtained if and only if the final variable cost of the activities is equal to the sum of the accounting expenses of the activities and the dual variable of calibration limit. So, the parameters of the cost function should be as follows:

$$\text{MC} = \frac{\partial C^V(X^0)}{\partial x} = d + QX^0 = C + \lambda \quad \text{Eq. 4}$$

Stage 3: Development of the final programming model in the third stage of PMP, we use a nonlinear calibrated cost function and the limitation of the resources to build a nonlinear programming model, as below:

$$\text{Max } Z = GM'X - d'X - \frac{X'QX}{2} \quad \text{Eq. 5}$$

s.t:

$$\begin{aligned} AX &\leq b \\ X &\geq 0 \end{aligned}$$

The solution for this final model in the base year's condition is the same as the activity level of the base year. It is possible to use the changes in the conditions and define different scenarios for analyzing the policies.

1. Positive mathematical programming models are widely used for analyzing and evaluating the adoptability of farmers to changes in the market and policies. In this research, this model is used for assessing its potential and its ability to actualize crop insurance plans. The model of participation possibility in the crop insurance plan is considered for every single crop. Here, the farmer participates in the program and pays his crop insurance fee. If the amount of his harvest is less than the expected rate, he will receive the calculated compensation based on the subtraction of the expected amount of the product from the real amount of the product (Forohideh and Tarazkar, 2009). In this case, the vector of expected gross returns and the covariance matrix of the gross return are recalculated, which is different from the insurance-less case. Therefore, the model is extended and the possibility is created to choose with or without a participation option in the suggested plan through the quadratic mixed-integer formulation. Therefore, based on the above-mentioned points, the final model of calibration used in this research will be as follows:

$$\begin{aligned} Z = GM'_{un}x_{un} - \frac{1}{2}\phi x'_{un} \sum_{un} x_{un} + GM'_{in}x_{in} \\ - \frac{1}{2}\phi x'_{in} \sum_{in} x_{in} - d'x - \frac{x'Qx}{2} \end{aligned} \quad \text{Eq. 6}$$

s.t:

$$Ax \leq b$$

$$x = x_{un} + x_{in} \quad \text{Eq. 7}$$

Limits:

$$x_{in} \leq b \times \delta \quad \text{Eq. 8}$$

$$x_{un} \leq b \times (1 - \delta)$$

2. Where  $x$  is the area under cultivation of wheat, barley, onion, watermelon, and melon in the field, and  $d$  and  $Q$  are the parameters of the quadratic cost function.  $\phi$  is the coefficient of risk aversion for the field. In this research, the coefficient of risk aversion from previous studies has been used.  $GM'_{in}$  and  $GM'_{un}$  are the expected gross return for wheat with and without insurance. The variable  $x$  is divided into two variables:  $x_{in}$  and  $x_{un}$ . These variables refer to the area of the field for cultivating wheat with or without insurance.  $\sum_{in} x_{in}$  and  $\sum_{un} x_{un}$  are the variance matrices of the gross return covariance of cultivating wheat with or without insurance. Sistan's limited resources are land, irrigated water, workforce, and investment.  $\delta$  is a special dual variable field that has two values, 0 and 1 (Vaderveer, 2001).

Calculated for the participation mode, the objective-oriented part is determined by the second and third lines of Equation 6. Based on the role of the insurance plan, the second line is calculated to determine the expected value and the covariance matrix of the gross margin. Equation 7 is limited if and only if the  $x$  variable series for wheat is equal to the variable  $x$  (with or without insurance). These limits provide the chance to select individual participants. In fact, when the variable  $\delta$  for a field equals 1, the farmer has to participate in the program with all other available fields, and vice versa. Therefore, for a field that participates in the program, ( $\delta = 1$ ), the objective function of equation 6 is eliminated, and the objective function just refers to the insurance mode. The opposite mode happens in a situation without insurance ( $\delta = 0$ ) (Schmid and Sinabell, 2005).

### 3. Results and Discussion

The population in the present study consisted of Sistani farmers who are classified into three subgroups:

- Small field with a cultivated area of less than 4 hectares.
- Average-sized fields with a cultivated area of between 4 and 10 hectares.
- Large fields, a cultivated area of more than 10 hectares.

The effect of accepting wheat insurance on the cultivation pattern and the gross return of the farmers

In this section, the impact of accepting insurance on the cultivation pattern of the farmers is analyzed (Table 1). First, the wheat insurance was introduced to all participants, and then its effects were processed by the PMP model. Table 2 shows the results of introducing this insurance to these three groups. As it can be inferred, in a small field, the cultivation areas of wheat, barley, onion, watermelon, and melon are 2, 0.5, 0.1, 0.3, and 0.2 hectares, respectively, and the total cultivated area is 3.1 hectares. Based on this table, after informing the farmer about the crop insurance and his acceptance of the program, his wheat cultivated area increased from 2 hectares to 2.01 hectares. In addition, his watermelon cultivated area increased from 0.3 hectare to 0.314 hectare, whereas the barley cultivated area decreased from 0.5 hectare to 0.492 hectare, the onion cultivated area decreased from 0.1 hectare to 0.095 hectare, and the melon cultivated area decreased from 0.2 hectare to 0.186 hectare. After participating in the wheat insurance program, his gross return increased from 18423290 Rials to 18511721 Rials, which is 0.479% more than his nonparticipation in the program. In an average-sized field, the cultivation areas of wheat, barley, onion, watermelon, and melon are 4.8, 1.5, 0.25, 1.2, and 0.5 hectares, respectively, and the total cultivated area is 8.25 hectares. Based on this table, after informing the farmer about the crop insurance and his acceptance of the program, his cultivated area of wheat increased from 4.8 hectares to 4.858 hectares, of onion increased from 0.25 hectares to 0.254 hectares, and of watermelon from 1.2 hectares to 1.227 hectares. Whereas the cultivated area of barley decreased from 1.5 hectares to 1.406 hectares, the

cultivated area of melon decreased from 0.5 hectares to 0.45 hectares. After participating in the wheat insurance program, his gross return increased from 48803550 Rials

to 49291580 Rials, which is 1% more than his nonparticipation in the program.

**Table 1. Number and percentage of sample farmers available for each group**

	The farmers of the area based on the cultivated farms		
	Small farm	Average-sized farm	Large farm
Total percentage of sample farmers	28.1	12.5	4.7
No. of samples	36	16	6
Size of sample farms	3.1	8.25	21.2

Source: research findings

In a large field, the cultivation areas of wheat, barley, onion, watermelon, and melon are 16, 1.5, 0.5, 2.2, and 1 hectares, respectively, and the total cultivated area is 21.2 hectares. Based on this table, after informing the farmer about the crop insurance and his acceptance of the program, his cultivated area of wheat increased from 16 hectares to 16.445 hectares, and of watermelon increased from 2.2 hectares to 2.266 hectares, whereas the barley

cultivated area decreased from 1.5 hectares to 1.128 hectares, of melon decreased from 0.5 hectares to 0.429 hectares, and of onion decreased from 1 hectare to 0.827 hectare. After participating in the wheat insurance program, his gross return increased from 139151140 Rials to 142421200 Rials, which is 2.35% more than his nonparticipation in the program.

**Table 2. The effect of accepting wheat insurance on the cultivation pattern and the gross return of the farmers**

crops	Small fam		Average-sized farm		Large farm	
	Current	Accepting insurance	current	Accepting insurance	Current	Accepting insurance
Wheat(not insured)	2	-	4.8	-	16	-
Wheat(insured)	0	20.1	-	4.858	-	16.445
Barley	0.5	0.492	1.5	1.406	1.5	1.128
Onion	0.1	0.095	0.25	0.254	0.5	0.579
Watermelon	0.3	0.314	1.2	1.227	2.2	2.226
Melon	0.2	0.186	0.5	0.45	1	0.827
Sum	3.1	3.097	8.25	8.25	21.2	21.195
Gross return	18423	18511	48803	49291	139151	142421

Source: research findings

As it can be observed, introducing the wheat insurance program to Sistani farmers has led to their total participation.

The reason for this comprehensive participation is the low rate of insurance fees paid for every hectare of this crop. This participation was accompanied by an increase in the wheat cultivation area, so their wheat cultivated area increased by 0.513 hectares more than the total under-cultivated area of wheat, i.e., a 2.25% increase. Moreover, after their participation in the insurance program, the gross return of the total farms increased by 3846517 Rials, which is 1.86 percent higher than the period before their participation.

When this program was introduced to the large farms of Sistan, the farmers accepted the insurance, and consequently, their wheat cultivated area and their gross return increased by 2.78% and 2.35%, respectively. The result of introducing the insurance program to the sample fields' participation was that they all attended the program and paid 64480 Rials/ hectare as the insurance fee. This participation was followed by an increase in the wheat cultivated area as well as the gross return of the fields so that the wheat cultivated areas of the small, average-sized, and large fields in Sistan were 0.56%, 1.2%, and 2.78% and their gross returns were 0.48%, 1%, and 2.35%, respectively. However, based on these findings, the following suggestions are presented:

The participation rate of farmers depends on the size of their fields. Smaller farms have a higher sensitivity to the supporting rates, so, in order to insure the crops, it is

suggested to divide farmers into homogeneous subgroups and their supporting rates be determined accordingly.

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## Investigating the morphological, nutritive, and secondary metabolite characteristics of *Calotropis procera* (Aiton) R. Br wild populations in certain habitats of Baluchestan, Iran

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### ABSTRACT

The development and implementation of effective, beneficial programs towards restoration, conservation, and appropriate utilization of medicinal plant species are feasible only through the identification of their ecological characteristics. This research was conducted with the objective of investigating the morphological, nutritive, and secondary metabolite characteristics of calotrope wild populations in certain habitats of Baluchestan, as a completely random nested design with three repetitions throughout four cities during February/March 2014. The edaphic properties of habitats were also assessed. The obtained data were compared at a 5% significance level via DMRT. The samples were collected from various regions, including the cities of (1) Iranshahr (Mand-e Bala and Sarzeh villages), (2) Chabahar (Negour and Nalent villages), (3) Nikshahr (Bandaan and Bent villages), and Khash (Baluchkan and central Khash), were compared. Results showed that the majority of the investigated characteristics were significantly different among cities and regions. In this research, Nikshahr city had the highest number of morphological characteristics. The investigated soil in Nikshahr city was more suitable regarding P and K, as compared to other regions. The plants grown in Nikshahr had the highest level of foliar N, Ca, K, and P, the highest level of floral K, and the highest level of P and K in the roots. Forty-two compounds were identified in the aerial organs and root extracts of calotrope. Among the active ingredients, 9-Octadecenamide acid with 8.3% in the aerial organ, Benzyl alcohol with 8.7% in the root, and 2,6,10-Trimethyl,14-ethylene-14-pentadecane with 27.2% in the stem-extracted latex had the highest content in this plant.

### 1. Introduction

Calotrope (*Calotropis procera*) is a sap-producing plant of the Asclepiadaceae family that grows in the arid and semi-arid regions of Asia and Africa (Rashmi and Arya, 2011). This plant is distributed throughout the Hormozgan, Baluchestan, and Khuzestan provinces of Iran. Calotrope is considered a rubber plant that reaches a height of 3-4 m. In traditional literature, this plant is referred to as Ashar, Akras, Madaar, Ashar and is known as Karag and Kark in

Baluchi language (Mirheidar, 1994). Calotrope latex is used by Baluchestan natives as an antidote for curing the effects of insect and harmful creature stings. All parts of calotrope have therapeutic properties. Calotrope leaves treat common cold and coughing, while its latex has antiseptic and laxative properties and is used for the treatment of toothache and scorpion sting, as well as fetal abortion. The plant roots are useful for treating coughs, dyspnea, and fever. Its flowers are sedative and nourishing

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(Dewan et al., 2000). In India, the different plant parts are used to treat stomach ulcers, tumors, and diseases of the spleen and liver (Kirtikar and Basu, 1935). The latex of this plant has toxic effects on insects and fungi (Cleverson et al., 2016; Ramos et al., 2014) and harmful microorganisms (Shobowale et al., 2013; Asma et al., 2019), and effectively heals colon inflammation (Vijay et al., 2019) and swelling and aching of joints (Dewan et al., 2000) as well. The aqueous extract of this plant contains an alkaloid, flavonoid, glycoside, terpenoid, tannin sterol, and saponin (Asmaa et al., 2019).

Genetic and environmental factors and their interactions are the most important factors affecting plants' secondary chemical compounds. The most important environmental factors that greatly affect the quantity and quality of plant active ingredients are light, temperature, irrigation, location height (Omidbeigi, 2005), and soil chemistry (Vaićciulyte et al., 2017). By investigating the effect of climate on certain flavonoids of the hawthorn shrub (*Crataegus* spp.), Hematti et al. (2007) showed that there are interaction effects between location and characteristics. They concluded that the amount of quercetin in all organs of hawthorn in Kelardasht, Mazandaran, was higher than that of Gorgan. Jelena et al. (2019) reported that according to geographical location, all samples of *Thymus pannonicus* collected from natural habitats contain a higher amount of essence in comparison to cultivated plants. In another investigation conducted through the extraction and measurement of flavonoid, kaempferol, and quercetin compounds in the petals of ten genotypes of Damask rose in western Iran, Jaymand et al. (2009) found that the best locations regarding the amount of kaempferol and quercetin were West Azerbaijan, Ilam, and Ardabil, respectively. Tajali and Khazaeipour (2002) investigated the effect of altitude on the total phenol content and flavonoids of the *Crataegus microphylla* plant and stated that this plant contained the highest amount of the mentioned compounds at 1000 m altitude, as compared to plants that had grown at low altitudes. By investigating the ecotype diversity of *Thymus daenensis* in Isfahan and Chaharmahal-and-Bakhtiari provinces, Karimi et al. (2011) found that altitude increase had a positive effect on the amount of thymol and had no significant effect on carvacrol. They reported the highest amount of thymol in

the Sheikh Shabān sample at an elevation of 2747 m above sea level, and the highest amount of carvacrol was that of the Larak sample at an elevation of 2370 m above sea level. Nutrient elements significantly affect each other, which eventually impacts plant growth and productivity (Facchinelli et al., 2001). By investigating the effect of environmental factors on the level of antioxidants and phenolic and flavonoid compounds in the walnut plant, Ghasemi et al. (2011) reported that the highest level of phenolic and flavonoid compounds was achieved at the highest altitude and lowest daily average temperature.

The environment is known as the most important factor affecting the expression level of secondary metabolite biosynthesis genes in medicinal plants (Saharkhiz, 2002). Dowling et al. (1986) reported that the coverage percentage of *Acacia heterophylla* increases by increasing the organic matter, N, S, K, P, exchangeable Ca, and soil depth. Therefore, recognizing the factors that influence the quality and quantity of medicinal plants' active ingredients is of great importance. Considering the aforementioned facts, the present study investigates morphological, nutritive, and phytochemical characteristics, as well as the soil properties of locations where calotrope are established, in certain natural habitats of Baluchestan, Iran.

## 2. Materials and methods

### 2.1. Site description

Sistan and Baluchestan Province includes Baluchestan in the south and Sistan in the north, with a 181785 km<sup>2</sup> area, twenty cities, and comprising over 11% of Iran. The Baluchestan region, with a total of 15 cities, comprises two districts of Sarhad (including Zahedan, Mirjaveh, and Khash cities) and Makran (including Saravan, Iranshahr, Nikshahr, Chabahar, Sarbaz, Konarak, Delgan, Mehrestan, Sib va Sooran, Qasr-e-Gand, Fanouj, and Bampur) (Figure 1).

To determine the distribution spots of *Calotropis procera* in Baluchestan, the habitat range of this plant was first identified using reference (Mozafarian, 2004), interviewing the Natural Resources Department experts of various cities, and field surveys. The majority of calotrope habitats are located in the Makran district in the southern region of Baluchestan.

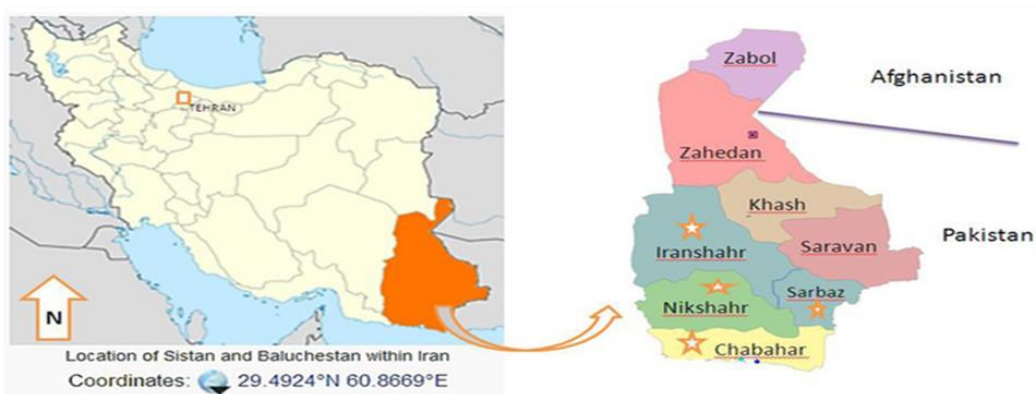


Figure 1. Geographical situation of Sistan and Baluchistan province, Iran. Studied cities are shown on the map

This research was conducted as a completely random nested design with three repetitions and four treatments (throughout four cities) during February/March (flowering season) of 2014. The study was carried out in Mand-e Bala and Sarzeh villages of Iranshahr city; Negour and Nalent villages of Chabahar city; Badaan and Bent villages of Nikshahr city; central region and Baluchkan Village of Khash city. To determine the soil properties of various habitats, soil samples were taken from zero to 60 cm depths and transferred to the laboratory. Leaves, flowers, roots, and calotrope latex were also collected by performing field surveys in various regions. Three plant populations were randomly sampled from each region, and their morphological characteristics were examined. Three leaves were collected from the mid-section of branches to assess leaf properties, and three inflorescences were collected from the distal part of the stem to assess flower properties.

To examine root properties, three profiles were dug out in the target habitats and three samples were collected from rootlets at 30-60 cm depths. Finally, all three organs, including root, leaf, and flower, were shadow dried and prepared for the experiments to determine element percentages. To collect the latex, several leaves were chosen from the mid-sections of the branch, and after detaching them from the branch, the latex excreted from the leaf was immediately collected in dark 10-milliliter glass vials. To measure the active ingredients of the organs, the samples collected from the two regions of each city were combined and sent to the laboratory. While collecting plant samples, a GPS device was used to record the geographical location of the region, including elevation from sea level, longitude, and latitude (Table 1), and the meteorological information of the studied cities was obtained from weather stations (Table 2).

**Table 1. Geographical location of the studied cities**

Row	City	Area in city	Longitude	Latitude	Altitude (m)
1	Iranshahr	Sarzeh	61° 42'	28°13'	560
2		Mand-e Bala	61° 38'	28° 06'	416
3	Chabahar	Negour	60° 10'	25° 42'	11
4		Nalent	61° 24'	25° 45'	10
5	Nikshahr	Bent	61° 31'	27° 16'	340
6		Badaan	61° 03'	27° 11'	432
7	Khash	Central Khash	61°21'	28° 21'	911
8		Baluchkan	61° 48'	28° 30'	815

**Table 2. Characteristics and average climate of the studied cities**

City	Climate	Av. An. Temp. (°C)	Av. An. Rain. (mm)	Av. An. Hum. (%)	Av. An. No. Frost Day	Av. An. No. Day. Above 35°	Av. Max. Da. Temp. (°C)
Khash	Semi-dry and moderately warm	20	153.0.	31	20	110	28.4
Iranshahr	Warm and dry	26.9	116.8	31	1	187	35
Nikshahr	Hot and dry desert	28	175.2	36	0	231	34
Chabahar	Hot and dry beach	26.3	114.3	76	0	13	30.2

## 2.2. Measurements

The soil texture was determined using the Hydrometer Method and via the Soil Texture Triangle (Klute and Dirksen, 1986). Thomas's method was implemented to measure soil pH (Thomas, 1996). To measure K and Na, saturated extracts were prepared from the soil samples, and K and Na were measured using a flame photometer device (model- CI361) (Mostofi and Najafi, 2005). Soil P was measured using the Olsen method (Olsen and Sommers, 1982), and N was measured via a Kjeldahl apparatus (model V40) (Page et al., 1982).

Morphological characteristics, including branch length, internode distance, leaf length, leaf width, inflorescence length, inflorescence width, number of leaves, and stem diameter were measured using equipment such as a scaled ruler and a caliper device.

The phosphorus content of plant tissues was determined using the digestion method and a spectrophotometric device (Sadou et al., 2007), the potassium content was determined using the flame emission method and a flame photometer device (Sadou et al., 2007), the nitrogen content was determined using the Kjeldahl apparatus (Page et al., 1982), and the other elements were determined using an atomic absorption apparatus (Baker and Amacher, 1982).

A gas chromatography device (Agilent 6890) was used to identify the active ingredients. The device consisted of a 30-m column with an internal diameter of 0.25 mm and a layer thickness of 0.25 µm of the BPX5 kind. To identify the constituent compounds of the essence, one µl of the sample that had been diluted by n-Hexane was injected into the GC/MS device. The temperature program of the column was set as follows: Initial oven temperature of 50°C and pause at this temperature for 5 minutes, a temperature gradient of 3°C per minute, increasing the temperature up to 240°C and then with a speed of 15°C per minute, increasing the temperature up to 300°C and a 3-minute pause in this temperature and the response time was 75 minutes. The injection chamber temperature was 290°C as a split of 1 to 35, and helium gas was used as the carrier gas with a flow speed of 0.5 mm per minute. The mass spectrometer (model Agilent 5973) had an ionization voltage of 70 electronvolts, the ionization method was EI, and the ion source temperature was 220°C. The mass scanning range was set at 40 to 500. Chemstation software was used. Spectrum identification was carried out using their retention index and comparing it to the indices available in reference books and articles, using the mass spectrums of standard compounds, and using the

information available at the computer library (McLafferty and Stauffer, 1989; Adams, 2004).

### 2.3. Statistical analysis

The data from this study were first tested for normality before being analyzed using a nested design with three repetitions. Data analysis was performed by SAS software (version 9.2), and means were compared via Duncan's multiple range test at a 5% significance level.

## 3. Results and Discussion

### 3.1. Soil edaphic properties of calotrope habitats

The edaphic properties of habitat soils investigated in this study included EC, pH, the amount of N, P, K, and Na, and the percentage of sand, silt, and clay. The level of pH,

EC, Na, clay, and silt in the soil did not reveal a significant difference between cities, but the impact of city regions on all properties of the soil of the calotrope shrubs was significant at a 1% level (Table 3). The calotrope habitat soil of the Bent region in Nikshahr city had the maximum level of K and P (Table 3). The highest level of N was measured in the Negour region of Chabahr city, and the highest level of Na was measured in the Mand-e Bala region of Iranshahr city (Table 4). The level of Na was higher in Iranshahr city. A possible reason for higher soil salinity in Iranshahr habitats may be the excessive usage of water in this city which is due to the excavation of deep wells for agricultural utilization, and water salinity results in soil salinity.

**Table 3. Analysis of variance of soil properties in different habitats of calotrope**

S.V.	df	EC	pH	N	P	K	Na	Sand	Clay	Silt
City	3	2.81ns	0.52ns	0.01**	1.62**	156.39*	0.027ns	96.66**	10.26ns	61.95ns
Area in city	4	1.86**	0.64**	0.007**	0.7**	79.18**	0.032**	168.86**	6.42**	147.69**
Error	24	0.00001	0.00001	0.000003	0.000003	0.001	0.0001	7	1.16	0.15
CV (%)	-	0.27	0.055	1.63	0.46	0.094	3.45	7.37	8.43	3.82

\*, \*\* and ns are significant at 5%, 1% and no difference, respectively.

**Table 4. Mean Comparison of soil properties in different habitats of calotrope**

City	District	EC (dS m <sup>-1</sup> )	pH	N (%)	P (ppm)	K (ppm)	Na (ppm)	Sand (%)	Clay (%)	Silt (%)
Iranshahr	Sarzeh	2.06 a	8.28a	0.14b	0.18 e	36.61c	155.77b	84.45a	10.815c	4.74e
	Mand-e Bala	2.38 f	7.06c	0.14b	0.21c	30.31f	156.07a	71.32d	12.18bc	16.50b
Chabahr	Negour	0.92d	8.18b	0.16a	0.11f	32.57e	108.91f	69.05d	15.11a	15.28c
	Nalent	0.93e	8.18b	0.14b	0.21d	33.17d	110.36e	78.75c	12.05b	9.04d
Nikshahr	Bent	1.62b	8.19b	0.07d	1.10b	41.12a	123.21c	83.28ab	13.14b	3.58f
	Bandaan	1.61c	8.18b	0.10c	1.05a	41.10 a	122.25d	80.10cb	12.51bc	7.39d
Khash	Central Khash	0.64g	8.18b	0.06e	0.15g	30.1g	98.11g	69.47d	12.26bc	18.27a
	Baluchkan	0.64g	8.19b	0.05f	0.09g	30.05h	98.23g	82.60abc	12.14b	5.26e

The common letters in each column indicate no significant difference.

Soil is among the most important environmental factors that play a major role in the distribution and density of plant vegetation. In fact, soil characteristics are the result of the effects of other environmental factors over a period of time. Soil types determine the plant species, and plants affect the nutrient element cycle and spatial properties of the soils (Kouchaki and Azizi, 2005). The highest amount of soil N was measured in the Negour habitat of Chabahr city, while the lowest amount of this element was measured in Khash city. One way of N removal from the soil is the leaching process (Tabatabaie, 2013). The highest amounts of K and P were measured in the Bent region of Nikshahr city. Considering the impact of soil texture on the absorption level of elements in the soil, the higher amounts of these two elements in the soil of Nikshahr city habitat can be attributed to the higher clay content in the soil of this city, such that the elements' positive charges are attracted by clay's negative charge. After evaluating the elements Zn, Cd, Na, Mg, K, Fe, and Ca in various species, Mohamed et al. (2003) concluded that the concentration level of elements differs in various species, as well as in similar species from different habitats. Ankita et al. (2016) also reported that the N, P, and K levels varied in different soil samples, and their amounts were in the ranges of 7.40–89.70 mg gm<sup>-1</sup>, 14.38–82.75 mg gm<sup>-1</sup>, and 0.76–5.46 mg gm<sup>-1</sup>, respectively.

The highest amount of Na was measured in the Mand-e Bala region of Iranshahr city. Jafari et al. (2006) found that special relationships exist between the type of plant and soil characteristics in the Howz-e Soltan region of Qom province, and the roles of salinity and texture were more influential than the other factors. Soil texture plays a role in the establishment of plant vegetation through the humidity level and the aeration of available nutrients (Jafari et al., 2002). Investigating some soil characteristics such as texture, EC, sodium adsorption ratio, organic matter, gravel percentage, and plant attributes such as density and canopy surface in 35 calotrope habitats in the southern pastures of Fars Province showed that the calotrope shrubs could grow in a wide range of soil conditions, such that up to 50% of surface gravel, up to 10 dS m<sup>-1</sup>, up to 8.56 sodium adsorption ratio, heavy, moderate to light textures, and soils with a low content of organic matter (0.1%) are not limiting factors for plant establishment (Sadeghian et al., 2010).

### 3.2. Morphological characteristics of calotrope in various habitats

The studied morphological characteristics of calotrope included branch length, internode distance, leaf length, leaf width, inflorescence length, inflorescence width, number of leaves, and stem diameter. A statistical difference was observed among all characteristics, except inflorescence

length, in the studied cities (Table 5). Differences in all characteristics were also significant at a 1% level among the various habitats (regions of a city). According to the

data presented in Table 6, Nikshahr city had the highest branch length, leaf length and width, inflorescence length, and stem diameter among all the studied cities.

**Table 5. Analysis of variance of morphological traits of calotrope in different habitats of Baluchestan mean of squares**

S.V.	df	Branch length	Intermediate distance	Leaf length	Leaf width	Flower length	Flower width	No. of leaves	Stem diameter
City	3	239.21**	15.5**	32.21**	13.02**	5.68 <sup>ns</sup>	2.63**	672.74*	139.6**
Area in city	4	119.63**	7.35**	30.65**	9.76**	10.56**	3.35**	806.53**	65.36**
Error	24	0.23	0.01	12.55	1.07	2.95	0.55	11.05	0.07
CV (%)	-	0.24	2.24	2.09	12.43	19.96	11.04	5.63	1.82

\*, \*\* and <sup>ns</sup> are significant at 5%, 1% and no difference, respectively.

**Table 6. Mean Comparison of morphological traits of calotrope in different habitats of Baluchestan**

City	District	Branch length	Intermediate distance	Leaf length	Leaf width	Flower length	Flower width	No. of leaves	Stem diameter
Iranshahr	Sarzeh	194.25e	7.0a	8.62e	5.45f	7.62c	6.89c	73.00a	16.85c
	Mand-e Bala	195.0d	7.12a	13.25b	7.51e	10.87a	8.11a	64.500b	15.75d
Chabahar	Negour	197.0c	3.97d	11.75 c	9.06c	8.0bc	6.98bc	63.0b	8.35e
	Nalant	198.25b	4.05d	8.62e	7.51e	8.75b	7.02bc	26.0d	7.80f
Nikshahr	Bent	200.25a	3.95d	15.87a	10.61a	7.37c	5.37d	63.75b	17.12a
	Bandaan	200.50a	3.92d	13.62b	8.23d	11.37a	7.10b	65.0b	16.89c
Khash	Central Khash	187.25f	5.05b	11.37dc	9.49b	7.62c	6.92bc	61.75b	17.02b
	Baluchkan	186.25g	4.6c	10.25d	8.89c	7.25c	5.40d	55.25c	16.89c

The common letters in each column indicate no significant difference

Regarding the majority of studied characteristics, Nikshahr city, with about 450 m of elevation above sea level, had the maximum measured values (Table 6). The calotrope species in Sistan and Baluchestan Province is distributed at 10-9110 m above sea level. One of the influential factors regarding the distribution or lack of distribution of plants is elevation from sea level. With the rise or decline of elevation, habitat conditions change, especially in terms of climate, and plants become established in an elevation range based on their ecological needs (Tabataba'i and Qasriani, 1992). Also, the amount of P and K nutrient elements in calotrope decreased with elevation, and this factor can also be a reason for the reduction of morphological characteristics in high altitudes. These results are similar to those of Mellati (1995) and Salarian et al. (2009) that reported a significant relationship between elevation above sea level and canopy density and percentage. According to meteorology data (Table 2), increased altitude leads to reduced temperature, such that mean annual temperature declined from 26.3°C to 20°C in the highest altitude. Since the growth and development of plant organs decreased in low-temperature conditions, many of the morphological characteristics investigated in this study such as branch length, leaf length, and inflorescence length and width decreased. Najjar Firozjaee et al. (2014) investigated the effect of altitude on morphological and biochemical properties of nettle (*Urtica dioica*) leaves in Mazandaran and Gulestan Provinces and stated that high altitudes decreased the morphological characteristics of nettle, while the phytochemical properties of nettle showed better performance in high altitudes. The increase of factors such as organic matter, N, S, K, P, exchangeable Ca, and soil depth increased the canopy percentage of *Acacia harpophylla* (Dowling et al., 1986). The variability of soil properties in pastures may be

effective on the better performance of soil in absorbing nutrient elements and promoting plant growth (Shukla et al., 2004) and these elements vary and differ depending on the type of plant and chemical properties of soil such as pH (Gilliam and Dick, 2010). Morphological characteristic identification is considered the first step of evaluating diversity and genetic structure (Zanella et al., 2011) and is also indicative of the environmental conditions and factors in each region and city. Pouyanfara et al. (2018) morphologically examined 65 samples from seven wild populations and six cultivated populations of *Melissa officinalis* L. from five provinces in Iran and concluded that there is great morphological diversity among the populations of different regions. The effect of habitat on morphological diversity of *Melissa officinalis* L. (Hadj Ali et al., 2012), *Plantago psyllium* (Shahriari et al., 2018), *Salvia fruticosa* Mill. (Peggy et al., 2020), *Glychiza glabra* (Esmaeili et al., 2020), and *Chamomilla recutita* L. (Ieva et al., 2020) has also been investigated. The higher number of most morphological characteristics in Nikshahr city may be due to the higher levels of K and P, as well as the percentage of sand in the soil texture of this city's habitats. The Baluchkan region of Khash city with 911 m above sea level has the least values regarding the majority of characteristics, and the necessity of tending to Nikshahr, Iranshahr, and Chabahar cities is emphasized for the preservation of these valuable genetic resources.

### 3.3. The percentage of elements in different organs of the calotrope in various habitats

The analysis of the variance table for element percentages showed that the Na, Ca, K, P, N content of leaf, flower, and root × city were significant at a level of 1%. Leaf P and flower K were not significant in the region × city interaction, whereas flower Na and root K were

Table 7. Analysis of variance of percentage of elements of different organs of calotrope in different habitats of Baluchestan

Elements	df	N			P			K		
		Leaf	Flower	Root	Leaf	Flower	Root	Leaf	Flower	Root
City	3	0.651010**	3.02881146**	0.42665313**	0.00082**	0.00058983**	0.00013128**	16065.40865**	3455.12448**	982.488646**
Area in city	4	1.05398**	1.01984063**	0.06050313**	0.00004847**	0.00007538**	0.00008434**	1220.53219**	512.36344**	123.102812*
Error	24	0.08166458	0.032111562	0.00493646	0.00002553	0.00000212	0.00000951	69.54073	293.65885	31.837187
CV (%)	-	14.30	10.51	10.53	16.86	6.70	10.71	4.13	6.88	8.99

\*, \*\* and \*\* are significant at 5%, 1% and no difference, respectively.

Table 7 (Continued)

Elements	df	Ca			Na		
		Leaf	Flower	Root	Leaf	Flower	Root
City	3	0.00117**	0.00018878**	0.00214275**	15362.12792**	3787.27917**	2334.546146**
Area in city	4	0.00012441**	0.00009878**	0.00016931**	667.01250**	259.02937*	303.051562**
Error	24	0.00002732	0.00000195	0.00000902	9.94562	71.77688	32.714271
CV (%)	-	6.00	7.40	10.53	3.01	11.34	9.51

\*, \*\* and \*\* are significant at 5%, 1% and no difference, respectively.

Table 8. Mean comparison of percentage of elements of different organs of calotrope plant in different habitats of Baluchestan

City	Area in city	N (ppm)			P (ppm)			K (ppm)			Ca (ppm)			Na (ppm)		
		Leaf	Flower	Root	Leaf	Flower	Root	Leaf	Flower	Root	Leaf	Flower	Root	Leaf	Flower	Root
Iranshahr	Sarzeh	1.42d	1.69dc	0.66c	0.03cb	0.01bc	0.031b	152.90e	236.30bc	52.50d	0.04a	0.026a	0.015f	159.32a	96.75a	89.72a
	Mand-e Bala	1.80cd	2.19ba	0.44e	0.03c	0.01c	0.022c	136.87f	258.77b	58.50dc	0.03b	0.022a	0.022e	132.02c	96.85a	73.90b
Chabahar	Nagour	2.38ab	2.07bc	1.13a	0.02ad	0.01de	0.024c	202.65c	256.80b	68.65b	0.02c	0.021a	0.056a	143.75b	78.67ba	68.20bc
	Nalant	1.50d	2.01bc	0.88b	0.01e	0.01e	0.025c	180.77d	254.60b	63.85bc	0.02c	0.017b	0.045b	133.10c	98.90a	61.20c
Nikshahr	Bent	1.70cd	2.70a	0.52e	0.04a	0.03a	0.036a	268.27a	279.70a	70.05b	0.03b	0.012c	0.033c	48.85f	55.47c	51.45d
	Bandaan	2.71 a	1.38dc	0.50e	0.03ab	0.02ba	0.032ab	233.62b	262.52a	83.37a	0.04a	0.024a	0.028d	70.15e	45.20c	33.95e
Khash	Central	2.42ab	0.70d	0.63cd	0.02ed	0.02dc	0.033ab	229.42b	228.85c	53.87d	0.02c	0.014b	0.008g	77.00d	63.80b	51.47d
	Khash	2.02cb	0.86d	0.55ed	0.02cd	0.02de	0.024c	206.95c	214.02d	50.77d	0.01d	0.011c	0.019e	72.35e	61.95bc	50.82d

The common letters in each column indicate no significant difference.

significant in the region × city interaction at a level of 5%. The remaining characteristics were significant at a 1% level in the region × city interaction (Table 7). The comparison of means for the level of elements in the four studied cities indicated that the highest amount of leaf N was measured in the central region of Khash, and the lowest amount was measured in the Sarzeh region of Iranshahr. In most cases,

plants dedicate more N to the leaves. One of the reasons for a higher amount of N in the leaves, compared to other organs, is increased photosynthesis under the conditions of reduced stomatal conductance and water availability (Kerkhoff et al., 2005). Nitrogen is mainly transferred via mass transfer, intermediated by transpiration. The highest amount of P and K in all three organs was measured in the

Bent region of Nikshahr city. The highest amount of leaf Ca was measured in the Bent region of Nikshahr and Sarzeh region of Iranshahr in equal amounts, whereas the highest amount of flower Ca and root Ca was measured in the Sarzeh region of Iranshahr and the Negour region of Chabahar, respectively. Also, the highest amount of leaf and root Na was measured in the Sarzeh region of Iranshahr, and the highest amount of flower Na was measured in the Nalent region of Chabahar. The lowest percentages of elements including, N, P, and K in all organs of calotrope were measured in Iranshahr city (Table 8). The observed differences of elements in various organs of calotrope, among the cities and the city regions may be due to the different ecological traits of the regions such as

temperature, humidity, elevation from sea level, plant growth stage, harvesting season of plant, and other soil-related and geographical factors. Harati Rad et al. (2017) reported that different habitat conditions resulted in a significant difference of Ca, N, K, Zn percentages, and soluble carbohydrate of colocynth seeds at a level of 1%, such that the highest amount of seed ash and carbohydrate was observed in Zabol city and the highest level of elements was observed in Iranshahr city. Investigating the *Dracocephalum moldavica* L. plant in 5 different habitats of Iran (Salmas, Urmia, Khoy, Maragheh, and Tabriz), Yousefzadeh et al. (2018) concluded that the highest level of N (2.3%) and P (0.22%) were measured in the flowering branches of this plant in Salmas city.

**Table 9. Chloroform extract of calotrope leaf leachate in different habitats of Baluchestan**

Row	Substance name	Peak No.	Retention time (Min)	Iranshahr	Khash	Chabahar	Nikshahr
1	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl) phenol	1	10.72	1.1	0.9	--	1.4
2	2-Methylene-1,5-pentanediol	2	12.81	1.6	2.2	2.7	4.6
3	2,6,10-Trimethyl,14-ethylene-14-pentadecne	3	13.16	23.1	20.1	27.2	14.5
4	Bicyclo[4.1.0]heptane, 7-butyl	4	13.42	1.5	1.9	2.6	5.1
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5	13.59	5.7	7.1	3.8	10.1
6	Nonanoic acid, 7-methyl methyl ester	6	13.83	3.1	2.6	1.7	0.9
7	5-Norbornene-2-carboxylic acid	7	14.10	1.4	0.9	3.6	1.1
8	Bis-(3,5,5-trimethylhexyl) ether	8	14.39	2.5	3.7	1	0.8
9	Methyl 9-octadecenoate	9	14.55	9.3	12.6	14.3	16.7
10	6-Octen-1-ol, 3,7-dimethyl	10	14.91	3.4	5.1	2	1.1
11	(+)-(1r,2r)-2,7,7-Trimethyl-3-oxabicyclo[4.1.1.]Octan-4-one	11	15.24	--	1.7	--	1.8
12	6(e),9(z),13(e)-Pentetriene	12	15.42	1.9	1.5	1	2.3
13	Beta-L-galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)	13	15.60	9.2	7.3	5.7	4.4
14	D-xylopyranose, 1,2,3,4-tetrakis-o-(trimethylsilyl)	14	15.83	4.3	2.9	2.7	3.5
15	Bis-(3,5,5-trimethylhexyl) ether	15	16.41	1.5	1.8	1.4	2.5
16	Phytol	16	16.82	4.8	3.5	4.1	5.6
17	1,2-Benzenedicarboxylic acid, diisooctyl ester	17	17.69	1	1.7	1.1	2
18	1-(2-Hydroxyethoxy)-pentadecane	18	17.93	4.1	2.7	1.5	1.1
19	2,6,10-Dodecatricenoic acid, 7,11-dimethyl-3-(trifluoromethyl)-, methyl ester	19	19.78	1.2	1.6	1.3	1.5
20	Squalene	20	26.02	1.7	1.9	1.1	1.6
21	alpha-Amyrin	21	35.73	8.3	7.1	5.5	7
22	beta-Amyrin	22	37.03	2.9	3.3	7.9	8.7
23	Oleanolic Acid	23	38.41	4.1	5.2	6.1	1.6

Referring to Table 4, it is evident that the calotrope habitat soil in Iranshahr has the highest level of EC and Na. A nutritive imbalance in saline soils is among the main reasons for the reduction of plant growth and productivity. Studying the effects of salinity on clover has shown that increased salinity results in the reduction of the total dry weight of shrub, N percentage, NUE, and K concentration, whereas Na percentage increases in the aerial organs. Furthermore, the amount of elements absorbed by plant roots from the soil varies depending on the type of plant and the chemical properties of the soil, such as pH (Gilliam and Dick, 2010). However, it should be noted that the deficiency of an element in the plant does not indicate the deficiency of that element in the soil; the same element may exist at adequate levels in the soil, but its absorption does not occur due to the lack of required ecological conditions. The soil of Nikshahr city is more alkaline, and the level of

its leaf K and Ca is higher than the other cities. The different results obtained from the studied species indicate variations in the absorption level of nutrient elements among the various organs of the studied species, such that the leaf has a higher absorbing capability for the majority of the investigated elements in comparison to the flower and the root.

#### 3.4. The impact of habitat on the active ingredients of various calotrope organs

To measure the active ingredients of various calotrope organs in each habitat, a combinational sample from the two regions of each city was assessed. Examining the ingredients with GC\MS showed that 42 compounds have been identified in the extract of aerial organs and root of calotrope. The main components of active ingredients in the ethanolic extract of aerial organs and root of calotrope

**Table 10- Percentage of active ingredients of ethanolic extract of shoots and roots of calotrope in Baluchestan habitats**

Row	Substance name	Peak No.	Retention time (Min.)	Aerial part				Root		
				Iranshahr	Khash	Chabahar	Nikshahr	Iranshahr	Chabahar	Nikshahr
1	Tyrantone	1	5.33	5.3	3.3	3.7	3.1	0.8	0.6	-
2	Hydroxy methyl -2,pentanone	2	5.74	-	-	2.6	1.9	4.1	2.9	5
3	2-methyl 1,3-Buten-2-ol	3	6.52	1.4	1	-	-	0.5	1.9	1.6
4	Alpha-Thujene	4	7.48	2.1	0.7	2.9	2	1.6	2.2	0.9
5	Nonanal	5	8.02	1.3	1.6	5.2	5.6	7.7	6.1	8.1
6	Benzyl alcohol	6	8.74	1.7	2.1	-	1.3	5	8.7	7.2
7	Butene-2,2,-dimethyl	7	9.55	6.5	4.7	6.8	7.7	2.1	3	1.8
8	Myrcene	8	10.61	2.2	1.3	2.5	0.9	4.7	2.9	5.5
9	Catechin	9	11.25	6.3	4.2	1.5	5.5	1	0.8	1.3
10	1,2-Benzen dicarboxylic acid	11	11.78	1.7	2.9	0.7	1.8	1.8	2.4	2
11	Alpha-Phellandren	11	12.15	2.1	3.7	2.2	1	2.2	1.7	1.3
12	Epicatechin	12	12.63	2.9	2.7	3.3	2.1	-	0.6	-
13	1-Dodecene	13	13.08	2.2	1.8	0.9	-	1.1	1.8	1.6
14	3-Hexanol	14	13.55	1.4	2.8	1.6	1.1	3.7	2.9	0.8
15	Fumaric acide	15	13.97	-	-	1.2	0.9	-	-	-
16	1,8-Cineol	16	14.30	1.1	3	2.2	1.3	2.1	2.6	3.6
17	1-Tetradecene	17	14.83	-	-	-	-	7.9	5.9	8.3
18	Hexadecanoic acide methyl ester	18	15.22	5.4	7.6	3.4	5.1	1.8	2.5	1.3
19	9-Octadecenoic acid	19	15.53	3.2	2.9	6.5	8.3	-	-	-
20	Ethyl-9,12-Octadecanoate	20	15.91	-	3.3	1.6	-	2.4	3.3	1.8
21	Alpha-D-glucopyranoside	21	16.20	2.2	1.1	0.8	1.4	2	1.5	2.7
22	1,2,3,4-Tetrakis-o-pentopyranose	22	16.63	1.8	4	6.3	2.6	1.7	2.1	1
23	1,3-Hexanediol,2-ethyl	23	17.04	-	-	1.1	0.7	1.5	-	-
24	Cyclohexanol-3-methyl	24	17.44	3.5	1.7	0.9	1.5	2	1.4	1.8
25	1-Octanol, 3-7-dimethyl	25	17.72	2.4	4.3	-	1.1	-	1.1	0.8
26	3-ethyl- 1-tetradecene	26	18.03	-	0.8	-	-	8.1	5.8	6.7
27	P-Cumaroyl hexose	27	18.80	0.9	1.7	1.1	1.6	1.7	2.2	1.4
28	Rutin	28	20.06	2.3	1.4	2.8	2.5	2.5	2.3	1.7
29	Tridecene	29	21.78	2.6	4.2	3.1	4.7	0.8	0.7	-
30	Isobutylnonane	30	22.26	1.4	2.2	1	-	-	2.1	1.7
31	3-Octadecene	31	22.31	2.1	1.8	-	1.3	2.9	1.2	1
32	Myrcetin	32	23.45	0.9	2.4	0.8	2.4	1.1	1.7	0.8
33	1-pentadecene	33	25.33	6	4.1	6.7	3.9	2.2	0.9	1.4
34	Pentadecanoic acid	34	26.24	3.8	-	-	5.8	4.3	6.7	7.1
35	Hepta decene	35	27.04	2.1	1.8	1.5	3.1	0.8	1.3	0.9
36	Luteolin	36	28.55	3	2.1	1	1.7	-	-	-
37	9-Octadecenamamide	37	30.6	4.6	5.7	3.9	6.5	1.2	1.7	1.4
38	Ethyl linoleate	38	31.11	2.8	1.3	2	1.7	2	1.8	2.7
39	Apigenine	39	31.74	3.1	2.7	1.8	1.1	1.4	2.1	1.5
40	Docosane	40	31.90	-	-	0.8	0.9	6.2	7.7	4.6
41	Kampferol	41	35.21	2.4	1.3	4.7	3.6	1.9	1.5	2.6
42	$\beta$ -caryophyllene	42	37.10	1.2	1.5	4.4	3.8	2.6	1.7	1.9

were nonanal (7.7% in root and 5.6% in the aerial organs, collected from the Nikshahr region), benzyl alcohol (8.7% in the root in Chabahar city), butene-2,2,-dimethyl (7.7%

in the aerial organs in Nikshahr), 1-tetradecene (7.9% in the root in Iranshahr and 8.3% in the root in Nikshahr), 9-Octadecenoic acid (8.3% in the aerial organs in

Nikshahr), 3-ethyl-1-tetradecene (8.1% in root in Nikshahr), and pentadecanoic acid (7.1% in root in Nikshahr).

Also, 23 compounds were identified in the latex extracted from calotrope leaves. Among the active ingredients, the amount of 2,6,10-trimethyl,14-ethylene-14-pentadecane measured in Chabahr, Iranshahr, Khash, and Nikshahr was 27.2, 23.1, 20.1, and 14.5, respectively (Table 9). Also, the methyl 9-octadecenoate measured at 16.7%, 14.3%, 12.6%, and 9.3% in Nikshahr, Chabahr, Khash, and Iranshahr cities, respectively, was the second compound identified with the highest percentage. Overall, the maximum amount of most active ingredients identified in calotrope latex in Nikshahr and Iranshahr cities was measured at an altitude of 411–560.

Edaphic factors play a major role in the production of plants' secondary metabolites (Ankita et al., 2016). Numerous reports have revealed the positive effects of soil N, P, and K on the level of the essence and active ingredients of various medicinal plants (Omer et al., 2014; Sharma and Kumar, 2012; Zheljaskov et al., 2012; Ankita et al., 2016; Xiao-Dan et al., 2018). A reason for Nikshahr's prominence regarding the majority of active ingredients may be the higher content of P and K in the habitat soil of this city. However, investigating the effect of *Salvia miltiorrhiza*'s secondary metabolite levels in some natural habitats of China with different topographies, Xiao-Dan et al. (2018) reported that the amount of active ingredients in this plant is more dependent on climatic factors rather than the quantity of soil micronutrients. Baghazadeh-Daryai et al. (2017) evaluated the Morphological and genetic variations of the Iranian 101 genotypes of Christ's-thorn (*Ziziphus spina-christi* (L.) Desf., Rhamnaceae from 12 habitats in the South of Iran. In total, 32 qualitative and quantitative characteristics were measured. Flavonoid quercetin and saponin content were measured for nine dominant populations. Maximum saponin content was related to the Shahdad sample (large leaves and fruits with least thorn density) with 2.6  $\mu\text{g ml}^{-1}$ . Maximum flavonoid quercetin content (3.66  $\text{mg g}^{-1}$ ) was related to the Jahrom sample. According to Table 10, the amount of active ingredient compounds of aerial organs and roots of calotrope differs in various habitats. For instance, with the increase of elevation from sea level, the amount of benzyl alcohol in the root decreases, and the highest amount of this substance has been obtained from Chabahr city with the lowest elevation from sea level. Also, the level of butene-2,2,-dimethyl and 9-octadecenoic acid in the aerial organs of calotrope in Nikshahr city is the highest, whereas the lowest level of these active ingredients has been measured in the aerial organs of calotrope in Khash city, which has the highest elevation from sea level. The impact of altitude on the quantity of essences and type of active ingredients differs in various plants. Zargar et al. (2016) studied the *Epimedium elatum* for its active principle content at different habitats of Kashmir Himalayas. The results showed that the content of active principles in leaves varies significantly between plants growing at different habitats. The Icarin and Icariside-II yield (per plant) of wild populations significantly increased with a decrease in

altitude of habitat. Investigating the level of active ingredients in six samples of *Litsea coreana* Levl. var. *Lanuginosa* collected from various habitats, Zhao et al. (2018) concluded that altitude has the most impact on the essence level in the leaves of this plant.

The level of a plant's active ingredients is directly related to its biosynthesis, metabolism, and biological activity, which are all dependent on environmental and climate conditions (Barimani, 2008). The difference in type and percentage of essence constituent components could be the result of genetic or non-genetic variations in response to environmental differences in habitat ecosystems. In each habitat, one or more active ingredients were at higher levels compared to other regions, and, in general, the maximum amount of most identified active ingredients in calotrope latex was measured in Nikshahr and Iranshahr cities at 411–560 m above seed level.

According to the results, the best growing location for this plant for achieving optimum quantitative and qualitative efficiency of active ingredients is at an elevation of 510 m above sea level. Among the important factors influencing essence components are temperature and humidity (Salehi Surmaghi, 2008), and the higher levels of certain compounds in Chabahr city may be due to the higher levels of humidity. Plant growing location may affect the process of active ingredient formation through changes in temperature and humidity. It has also been reported that the quantitative and qualitative diversity of chemical substances in *Helichrysum italicum* ssp. *Microphyllum* is not only dependent on differences in habitat (mountainous and coastal) but also dependent on local events (Melitoa et al., 2016). Laurel et al. (1999) also reported that the highest level of hypericin accumulation in perforated St. John's-wort (*Hypericum perforatum*) is achieved when plant growth and production occur in regions with high relative humidity.

The higher levels of certain plant chemical compounds such as octadecanoate and alpha-phellandrene in altitudes of above 900 m indicate the plant's tolerance to cold and adverse conditions of higher altitudes and its capability of producing an optimal amount of certain medicinal active ingredients. Although in case the goal is to harvest a high percentage of active ingredients, it should be considered that selecting the plant from high altitudes and extremely cold regions is not suitable for the optimal production of active ingredients. In the present study, the difference in highest and lowest elevations from sea level in the eight studies habitats is 901 m. Hadian et al. (2011) studied on the essence obtained from the plants of 18 landraces of *Zataria multiflora*, collected from various regions of Iran, also showed that this plant's essence contains 56 compounds and the effect of habitat on the percentage of active ingredients was significant.

#### 4. Conclusion

The effect of environmental factors on the absorption rate of substances by plants, morphology, and production of active ingredients in medicinal plants is very complicated and ambiguous, and the interpretation of results must be carried out with proper delicacy. All four

studied cities possess the required conditions and favorable weather for the growth of calotrope plants. The maximum amount of the most active ingredients identified in the leaf latex of calotrope, which has the highest medicinal applicability in the Baluchestan region, was measured in Nikshahr and Iranshahr cities. The results of this study indicate that calotrope habitats in Nikshahr city, with an average elevation of 450 m above sea level and higher contents of P and K, are considered the primary habitats of this plant in Baluchestan.

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## Changes in morpho-physiological characteristics of peppermint by foliar application of biofertilizer and nanofertilizers

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### ABSTRACT

Peppermint is one of the medicinal and aromatic plants that is widely used as a pharmaceutical, food, and cosmetic. In recent years, due to the harmful effects of fertilizers on the environment, their use has been criticized. Using bio and nano-fertilizer as an alternative source for conventional fertilizers leads to the gradual realization of fertilizer nutrients in the soil and in a controlled manner. The use of these fertilizers leads to increased nutrients, use efficiency, reduce the toxicity of the soil, and reduce the frequency of the application. The current study evaluates the effect of nano-fertilizers and organic fertilizers on morpho-physiological characteristics of peppermint based a completely randomized design. Experimental treatments were the foliar application of nano fertilizers (nitrogen, iron, and potassium) and normal vermiwash, enriched vermiwash, tea compost, and control. The results showed that the effect of nano-fertilizers and bio-fertilizers on plant height, leaf area, leaf dry weight, total dry weight, photosynthetic pigments, proline, protein, and carbohydrate were significant. All treatments increased photosynthetic pigments, compared to control. The maximum amount of proline, total dry weight, and plant height was obtained using nano fertilizers of nitrogen whereas enriched vermiwash resulted in the highest protein and leaf area.

### 1. Introduction

Mint is among those medicinal plants, which, due to medicinal effects and alimentary use, have been of interest to researchers since a long time ago. It has been used as spice and medicine since two thousand years ago. However, the use of peppermints (*Mentha piperita* L.) by humans started only 250 years ago. Leaves, vegetative parts, and essential oil of this plant have been cited as medicines in the most reliable pharmacopeias. Active ingredients of peppermints are used in food, cosmetics, pastry, soft drink, and spice industries (Omidbeige, 2006). This plant is among those valuable medicinal plants, the effects of which have been shown for the prevention and treatment of irritable bowel syndrome, based on recent studies (Izadi et al., 2009). Peppermints has medicinal properties and many applications, such as anti-gas and antibacterial medicines and is used as a constituent of medicinal plants produced in Iran, such as Altadine,

Masoument, mint drop, Menta, and Alicom (Omidbeigi, 2006).

Nanotechnology is a science and technology which has recently attracted much attention. This technology, which is an approach in every field of study, has the ability to produce modern materials, tools, and systems by manipulation of atomic and molecular levels (Lane, 2001). One of the most important applications of nanotechnology in different agricultural fields in the water and soil part, is the use of nano fertilizers for plant nutrition. Nowadays, biofertilizers are considered an alternative for chemical fertilizers, with the goal of increasing soil fertilization and producing yields in sustainable agriculture (Wu et al., 2005). Biofertilizers contain living cells of different microorganism types, which prepare the conditions for plants to absorb nutritive elements through biological processes (Han et al., 2006). Usually, these microorganisms cause the production of compositions such

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as gibberellin, cytokinin, and auxin, facilitation of the absorption of water and nutritive elements, especially phosphorus, nitrogen, and microelements from the soil, and disease reduction or prevention in plants (Hayat, 2010). The difference between biofertilizers and organic and chemical fertilizers is that they don't directly provide any nutritive elements for the plant (Han et al., 2006).

Vermiwash is a liquid containing plant growth stimulants, collected after the water has moved through a column of active worms (the worms used in the production of vermicompost) and can be used as a foliar application on plant leaves. Enriched vermiwash is obtained by a mixture of normal vermiwash enriched with aquarium water and bioreactor with extracts from Nettles and *aloe vera* plants, and its application, in addition to having positive effects on economic aspects and better performance, helps bioenvironmental sustainability. The extract obtained by the solution of water and vermicompost is called compost tea and is considered one of the best inputs in organic agriculture.

The results of one study have shown that the essential oil percentage of the medicinal plant peppermint for the *Azotobacter* and *Azospirillum* treatment is equal to those of the treatments with chemical fertilizer application (Kalra, 2003). In a study by Mehrafarin et al (2011), the results indicated that the fertilizer treatments had a significant effect on the performance of medicinal compositions of the plants, including the amounts of the essential oil, menthone, menthol, and biofertilizers significantly increased the amounts of the essential oil, menthone and menthol compared to the control treatment. Abdu and Mohammad (2014) reported that the consumption of 46 t/ha of compost increases the performance of the dry weight and the wet weight by 43 percent, compared to common complete fertilizers. In a study, the possibility of using biofertilizers instead of chemical fertilizers was investigated for the plant fennel, and the results were indicative of an increase in the vegetative growth, the performance, and the amounts of essential oil for biofertilizer treatments (Azzaz et al., 2009). In another study, the results of biofertilizer consumption by Ajwain (*Carum copticum*) showed the significant effect of biofertilizers on all of the characteristics. The highest biological performance, seed performance, essential oil performance, and content were obtained by the treatment seed inoculation + foliar application in the stem elongation stage (Ghilavizadeh et al., 2013).

Since the global approach in the production of medicinal plants is towards the improvement of quantity and quality of active ingredients, the application of nano fertilizers and biofertilizers is examined in this study.

## 2. Materials and Methods

This study was performed as a pot experiment. For this purpose, firstly the peppermint rhizomes were divided into parts with 3 nodes and transported to plastic pots with crater diameters of 20 cm and lengths of 18 cm, the soils of which contained farm soil and sand and vermicompost with the ratio 1:1:2. The experiment was performed as a completely randomized design with 7 treatments and 3 replications.

The plants were regularly watered with normal water. 3 weeks after the plants had reached the appropriate growth, the stems were headed back from a height of 5 cm. Then, the fertilizer treatments, including nitrogen nanofertilizer, iron nanofertilizer, potassium nanofertilizer (with the concentration of 2%) and normal vermiwash, enriched vermiwash, compost tea (with the concentration 1 in 3 water) and the control, were applied as foliar application in 4 repetitions during a course of 8 weeks.

### 2.1. Characteristics under investigation:

Measurement of the photosynthetic pigments: To do this, the Arnon (1949) method was used. For this purpose, 0.1 of the fresh weight of the leaf was ground using 10 ml of 80 percent acetone in porcelain mortar and, then, the solution was moved through a filter paper. The filtered solution became ready for examination by a spectrophotometry device of JENUS UV-1200 model. After the calibration of the device, each sample was examined for three wavelengths of, 663 nanometers for the chlorophyll a examination, 645 nanometer for the chlorophyll b examination and 470 nanometer for the examination of carotenoids and xanthophyll. The numbers relating to the absorptions were put to the following formulae and the amounts of the chlorophyll a and chlorophyll b and the sum of carotenoid and xanthophyll were calculated using the suggested equations by Arnon (1949).

The measurement of amino acids in leaf proline was performed by using Bates et al (1973) method. For this, 0.5 grams of the sample was ground using liquid nitrogen in porcelain mortar. Then, 10 ml of 3 percent sulfo salicylic acid was added to the sample and it was ground again. The resulting solution was placed for 15 minutes in a 10000 revolutions per minute centrifuge. 2 ml of the liquid was taken from the surface of the centrifuged solution and, under the hood, 2ml acetic acid and 2ml of ninhydrin reagent (1.25 gram ninhydrin + 30 milliliters of glacial acetic acid + 20 milliliters of 6 Molar phosphoric acid) were added to it and it was mixed for 15 minutes at 40 degrees centigrade by the mixer. The solution obtained was placed in a bain-marie of 100 degrees centigrade for 1 hour. Then, it was placed in a cold water bath for 10 minutes and, then, 4 ml of toluene was added to it. The tube contents were intensely mixed to obtain two phases. In order to read the absorption amount in the spectrophotometry device, toluene was used as the control, and in order to measure the proline amount, the absorption amount of the top phase was measured in the wavelength of 520 nanometers. In order to measure the protein concentrations, one gram of plant tissue and one milliliter of 50 milli molar tris-hcl extraction buffer with a pH of 7 containing 10 mM MgSO<sub>4</sub>, 2 mM EDTA, 20 mM DTT, 10% glycerol (V/V) and 2% PVP (V/W) were ground in the mortar and, then, the solution was transferred to the centrifuge tube and centrifuged at 4 degrees centigrade for 20 minutes in 13000 g by a refrigerated centrifuge machine. Then, the floating solution was collected and, once again, centrifuged for 20 minutes in 13000 g and, then, transferred to an eppendorf. The volume of the resulting supernatant was recorded and it was placed for short term preservation at 70 degrees centigrade.

The resulting protein extracts were used to measure the protein. In order to measure the quantity of the protein existing in the extracted protein extracts, the Bradford (1976) was used. To measure the soluble sugar (Kochert, 1978) 0.1 grams of dried leaves plus 10 milliliters of 70% ethanol were placed in a refrigerator for one week, so that its soluble sugars dissolve. Then, samples of 0.5 ml were taken from the above mentioned solution and their volumes were increased to 2 ml by the addition of distilled water, and 1 ml of 5% phenol was added to them and they were

mixed by a vortex. Thereafter, 5 ml of concentrated sulfuric acid was added to every one of them under pressure. After half an hour, the wavelength 485 was used by the spectrophotometer device to measure glucose. The sugar quantities of the solutions were evaluated using the standard curve based on mg. kg DW<sup>-1</sup>. Also, in this experiment, the characteristics leaf area (by leaf area meter device), plant height, dry leaf weight and the number of lateral branches were evaluated.

**Table 1. Mean comparison for investigating the effect of different treatment proline and photosynthetic pigments of Peppermint**

Treatment	Proline (µM/g)	Carotenoids (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll a (mg/g FW)
Nano fertilizers of nitrogen	0.25 b	0.222 b	1.09 b	2.02 b
Nano fertilizers of potassium	0.054 d	0.196 d	0.877 e	1.82 e
Nano fertilizers of iron	0.104 d	0.197 d	0.91 d	1.86 d
Tea compost	0.814 d	0.194 d	0.878 e	1.8 f
Normal vermiwash	0.125 c	0.207 c	0.94 c	1.87 c
Enriched vermiwash	0.145 a	0.229 a	1.12 a	2.04 a
Control	0.158 e	0.180 e	0.78 f	1.7 g

Means followed by the same letter(s) are not significantly different at 0.05 level of probability

## 2.2. Statistical analysis of the data

The statistical analysis of the data was performed by using the SPSS software and the mean comparison of the data was done by using the Duncan test.

## 3. Results

### 3.1. Effects of nanofertilizers and biofertilizers on photosynthetic pigments

Based on the results, the experimental treatments had significant effects on the amounts of the chlorophylls a and b at 5% level. All of the experimental treatments caused increases in the amounts of the chlorophylls a and b and the highest increase was observed for the enriched vermiwash treatment, with which the amount of the chlorophyll a has increased by 20 percent compared to the control, and the amount of the chlorophyll b has increased by 43 percent. Following the enriched vermiwash, nitrogen nanofertilizer had the greatest effect, with an 18 percent increase of the chlorophyll a and a 39 percent increase of the chlorophyll b (Table 1).

Based on the results, the experimental treatments were significant on the carotenoids amount at 5% level. The greatest effect compared to the control was related to the experimental treatment enriched vermiwash, which had a 37 percent increase compared to the control, and the other experimental treatments had less increasing effects than the control (Table 1).

### 3.2. Effects of various nanofertilizers and biofertilizers on leaf proline content

Experimental treatments had significant effects on the synthesis amount and proline accumulation in the peppermint plant at 5% level. Except for the experimental treatment nitrogen nanofertilizer, which had the highest positive effect and caused a 60 percent increase in proline compared to the control and its difference from the control was significant, all of the other experimental treatments had negative and decreasing effects compared to the control, and the least negative effect was related to the experimental

treatment potassium fertilizer, with a 66 percent decrease in proline compared to the control (Table 1).

### 3.3. Effects of various nanofertilizers and biofertilizers on leaf soluble sugars

The results showed that, the effects of experimental treatments on the amounts of carbohydrates, which were reductases of peppermint leaves were significant at 5% level. The experimental treatment normal vermiwash had the greatest positive effect on the amounts of reductive carbohydrates and caused a 23 percent increase compared to the control, and the least positive effect was for the experimental treatment enriched vermiwash, and the other experimental treatments had negative effects compared to the control. The experimental treatment nitrogen nanofertilizer had the greatest negative effect and caused a 44 percent decrease in reductive carbohydrates compared to the control (Figure 1).

### 3.3. Effects of various nanofertilizers and biofertilizers on leaf soluble sugars

The results showed that, the effects of experimental treatments on the amounts of carbohydrates, which were reductases of peppermint leaves were significant at 5% level. The experimental treatment normal vermiwash had the greatest positive effect on the amounts of reductive carbohydrates and caused a 23 percent increase compared to the control, and the least positive effect was for the experimental treatment enriched vermiwash, and the other experimental treatments had negative effects compared to the control. The experimental treatment nitrogen nanofertilizer had the greatest negative effect and caused a 44 percent decrease in reductive carbohydrates compared to the control (Figure 1).

### 3.4. Effects of various nanofertilizers and biofertilizers on the leaf protein content

Experimental treatments had significant effects at 5% level on the synthesis amount and protein accumulation in the peppermint plant leaves. The experimental treatment

enriched vermiwash had higher protein amount compared to the control and caused a 7 percent increase. There was no significant difference between the experimental treatment nitrogen nanofertilizer and the control and the other treatments had decreasing effects compared to the

control and the lowest negative effects were obtained for the fertilizing treatments potassium nanofertilizer and compost tea with, respectively, 15 and 17 percent decrease compared to the control, and there was no significant difference between these two treatments (Figure 2).

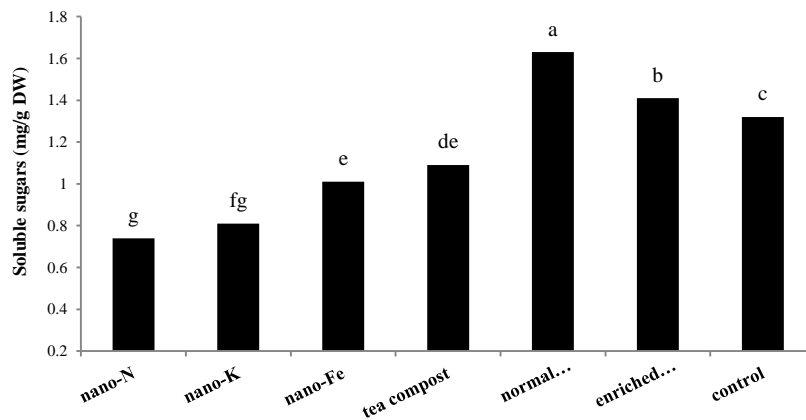


Figure 1. Effects of experimental treatments on leaf soluble sugars

### 3.5. Effects of various nanofertilizers and biofertilizers on the leaf area

Based on the results obtained from the variance analysis, the experimental treatments had significant effects at 5% level on the peppermint leaf area. The leaf areas of the experimental treatments enriched vermiwash and nitrogen

nanofertilizer were higher than the control, with respectively 26 and 16 percent increase in the leaf areas compared to the control, and the other treatments had less leaf areas compared to the control and the least leaf area was for the compost tea treatment, with a 23 percent decrease compared to the control (Table 2).

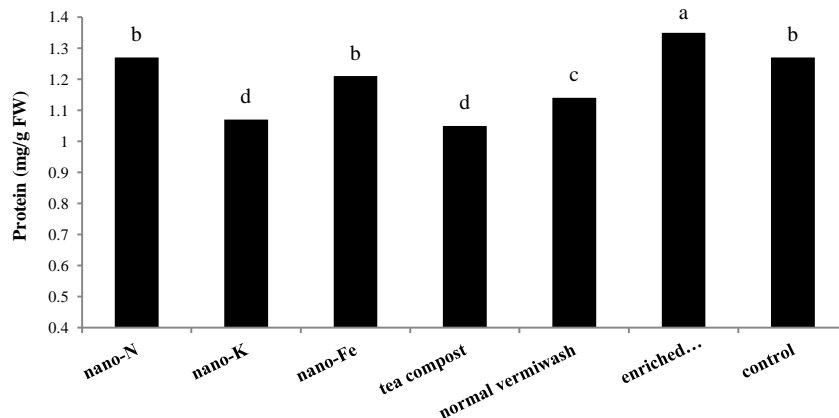


Figure 2. Effects of experimental treatments on leaf protein content

### 3.6. Effects of various nanofertilizers and biofertilizers on plant height

The results of variance analysis showed that the effects of various treatments on the peppermint stem length were significant at 5% level. All of the experimental treatments had increasing effects on the plant height compared to the control, and the highest plant height compared to the control was for the nitrogen nanofertilizer treatment with 139 percent increase, and the normal vermiwash treatment didn't have a significant difference with the control (Table 2).

### 3.7. Effects of various nanofertilizers and biofertilizers on total dry weight

Based on the results obtained from the variance analysis, the experimental treatments had significant effects at 5% level on the peppermint total dry weight. Except for the experimental treatment compost tea, all of the treatments had increasing effects compared to the control, and the highest amount of total dry weight was for nitrogen nanofertilizer, with 190 percent increase, and the least total dry weight compared to the control was for the compost tea treatment, with a 7 percent decrease (Table 2).

### 3.8. Effects of various nanofertilizers and biofertilizers on the number of lateral branches

Based on the results obtained from the variance analysis, the experimental treatments had significant effects at 5% level on the number of lateral stems in the peppermint. All

of the experimental treatments had positive effects compared to the control, and the highest number of lateral stems compared to the control was for nitrogen

nanofertilizer, with 146 percent increase, and the least positive effect was for the compost tea treatment, with a 10 percent increase compared to the control (Table 2).

**Table 2. Mean comparison for investigating the effect of different treatment on some morphological traits of Peppermint**

Treatment	Plant height (cm)	Leaf area (mm <sup>2</sup> )	Number of lateral branches	Dry weight (g)
Nano fertilizers of nitrogen	28.8 a	7846.2 a	60.7 a	4.59 a
Nano fertilizers of potassium	12.67 c	6521.6 b	29.7 cd	2.01 c
Nano fertilizers of iron	12.6 c	5385.8 c	37.0 bc	3.37 b
Tea compost	12.8 c	5157.4 c	27.3 cd	1.82 c
Normal vermiwash	12.0 c	6147.7 b	33.3 bcd	2.31 c
Enriched vermiwash	20.4 b	8545.3 a	40.7 b	3.40 b
Control	12.06 c	6736.6 b	24.6 d	1.73 c

Means followed by the same letter(s) are not significantly different at 0.05 level of probability

#### 4. Discussion

Overall, the results of this experiment showed that the application of concentrated vermiwash and nitrogen nanofertilizer has been beneficial for most of the characteristics under study and has improved them. The chlorophyll, the carotenoids and the leaf area were among these characteristics. The chlorophyll amount in living plants is one of the important factors for preservation of the photosynthesis capacity (Jiang and Huang, 2001). One study showed that the application of high rates of vermicompost resulted in increase in the leaf area, receiving more light and increase in photosynthesis (Sallaku *et al.*, 2009). Besides nitrogen, which causes an increase in chlorophyll amount and photosynthesis in plants, vermicomposts, as well, cause an increase in chlorophyll amount and photosynthesis in plants, because of having micronutritive elements, and especially iron. According to the report by Sairam *et al* (1998), carotenoids, by using the xanthophyll cycle and with epoxidation and de-epoxidation reactions, reduce the oxygen consumption and protect chlorophylls against photooxydation (Ansari 2008). Increase in leaf area determines photosynthesis capacity of plants. Change in leaf area, which is affected by genotypes, plant density, weather and soil fertility, will also affect the performance (Nezarat and Gholami, 2009). Yasari and Patwardhan (2007) stated that the amount of increase in the leaf area determines the photosynthesis capacity of the plant. These researchers emphasize the significant increase in leaf area measure with simultaneous applications of nitrogen fertilizers and biofertilizers. Using biological nanofertilizers has improved the growth of this plant. Better growth and higher performance of plants are attributed to slow release of nutritive elements with gibberellin and auxin due to application of biofertilizers like vermiwash. Soluble sugars act as osmoregulators, cell membrane stabilizers and agents of turgor pressure maintenance (Slama *et al.*, 2007). Hormones such as cytokinin, auxin, amino acids, organic acids, vitamins, enzymes such as protease, amylase, urease, secretions and mucoid materials from earthworm body and heterotrophic bacteria, fungi, actinomycetes, nitrogen fixing bacteria (*Azotobacter*, *Rhizobium*, *Agrobacterium*) and some phosphate solubilizing bacteria are also present in vermiwash (Shivsubramanian and Ganeshkumar, 2004). Asgari *et al* (2012) reported in a study that the growth

stimulant bacteria treatment had the highest plant growth among the experimental treatments (growth stimulant bacteria, vermicompost fertilizer and humic acid) for peppermint. Therefore, applications of bacteria, vermicompost and humic acid have resulted in a significant difference through the creation of better nutritive conditions for vegetative growth and the production of higher plants.

In the study performed by El-Gendy (2006) on basil, it was observed that the effect of nitrogen on the plant growth was significant and caused an increase in height compared to the control. Asgari *et al* (2012) reported that the experimental treatments growth stimulant bacteria, vermicompost and humic acid had significant effects on the dry weight of peppermint leaf and the growth stimulant bacteria treatment had the highest leaf dry weight. The plant dry weight is considered as an important indication of plant growth condition. Increase in the plant dry weight was indicative of more success in photosynthesis of the plant due to the availability of a more appropriate growth condition. The research showed that, through increasing the leaf area and providing the appropriate conditions for energy absorption and, also, participating in the structures of the enzymes involved in the photosynthetic carbon metabolism, the fertilizer causes an increase in photosynthesis efficiency and the essential oil performance in peppermint (Arabci and Bayram, 2004). Francis *et al* (2000), as well, showed that, with an increase in the nitrogen amount, the amount of the dry matter produced by chamomile increased due to volume increase of the plant canopy, the leaf area increase and more light absorption.

Many nanomaterials have a higher toxicity than ordinary materials. Nanomaterials are highly reactive, and the studies showed that they can result in oxidation causing harm to the cells, and they sometimes cause the deaths of cells and laboratory animals. Overall, the nanoparticle effects on living systems has not been examined sufficiently (Wang *et al.*, 2006). Whereas, sustainable agriculture based on biofertilizer consumption, with the aim of eliminating or markedly decreasing chemical input consumption, is considered a desirable solution for these problems. Biofertilizers contain conservatives with a dense population of one or more types of helpful organisms living in soil or in the form of their metabolic products, which are used to improve soil fertility and appropriately provide

nutritive elements required by a sustainable agriculture system, and, taking into account the positive effect of vermiwash on peppermint characteristics under study, can be used as an alternative for chemical fertilizers.

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## The first report of essential oil composition of *Vitex agnus-castus* L. growing in the Sistan region and its antibacterial activity

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### ABSTRACT

This study aimed to investigate the chemical compounds in the essential oil extracted from the flowering branch of *Vitex agnus-castus* L., grown in the Sistan region (Sistan and Baluchestan Province), and its antibacterial effects. Samples were hydrodistilled in a Clevenger-type apparatus and analyzed with gas chromatography mass spectrometry (GC-MS). In vitex essential oil, 30 bioactive compounds were identified. The most important compounds are 1,8-Cineole (39.16%), Sabinene (8.78%),  $\beta$ -Myrcene (6.44%), Sclareol (4.3%), and *trans*-Caryophyllene (3.17%). The composition of the essential oil of *Vitex agnus-castus* was described for the first time from Sistan region. *Bacillus cereus* (ATCC 11778) was the most sensitive strain against this essential oil, and *Pseudomonas aeruginosa* (ISIRI 275) was the most resistant strain. The monoterpenes and sesquiterpenes confer the chemical profile of the analyzed essential oil of vitex causing antibacterial effects. Further studies are required to explain the oil mechanism of action of this species involved in antimicrobial activities.

### 1. Introduction

Since 2000 years ago, the vitex plant has been used in the traditional medicine of different countries. The vitex genus of the Verbenaceae family grows wild in Iran and had four species of shrubs in the flora of Iran, including *V. trifolia* L., *V. negundo* L., *V. agnus-castus* L. and *V. psendoneggundo* is often observed in tropical and subtropical regions of the country, especially Sistan and Baluchestan, Khuzestan, Alborz (Karaj), Tehran, Qom, Khorasan, Hormozgan (Bandar Abbas), Lorestan provinces and different areas of the Persian Gulf (Nasri et al., 2005). VAC (*Vitex agnus-castus*), has been used since ancient Greek times as a treatment for menstrual problems (Webster et al., 2011). In addition, it has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction (Webster et al., 2006).

The insecticidal property of the plant has also been reported (Tandon et al., 2008). New research shows the therapeutic effect of chaste extract in premenstrual syndrome (PMS), elimination of menopausal

complications and increased milk production in lactating mothers (Eryigit et al., 2015). Vitex, Chaste tree, chaste berry, monk's pepper, and Abraham's balm are the latin names by which this plant is known. This plant is a tiny deciduous shrub that grows to be 1-2 meters tall, with a strong black pepper scent emanating from all parts. The plant has compound claw leaves (5 - 7 leaflets), dark greyish-green, long peduncles, slender flower clusters, purple bluish flowers and small berry fruits like black pepper. The leaves, flowers, and fruits of this plant contain active ingredients such as iridoid glycosides (acobin and agnoside), alkaloids (viticin), flavonoids (vitexin and castin), steroid hormones (progesterone and testosterone), and essential oil (1, 8- cineole and linalool) (Ghorbani Ghoushdi et al., 2021). Two glycoside compounds in the leaves of this plant have been identified as vitexin and vitexinin (Maltaş et al., 2010).

One of the most important secondary metabolites produced by this plant is essential oil. Essential oils are generally extracted using distillation, and often using

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steam. Essential oils extracted from different parts of a plant may differ in physical, chemical and odor properties. The constituents of essential oils change under different conditions. Therefore, it is not possible to comment definitively on the essential oils of a plant without considering specific environmental conditions. Essential oils are directly related to the biosynthesis, metabolism and biological activities of the plant which are a function of the climatic conditions of the plant environment. These oils are produced by plants as secondary metabolites for protection against bacteria, viruses, fungi and pests (Rota et al., 2008; Ghannadi et al., 2012). The antimicrobial properties of vitex essential oils and their constituents have been evaluated and reviewed (Malesand and Blazevic, 1998; Hamid et al., 2010; Küçükboyacıand and Sener, 2010; Ghannadi et al., 2012). Since the identification of the chemical components of plant essential oils from aromatic plants is needed to take better advantage of their opportunities, this study aimed to identify the secondary metabolites in the essential oil of the vitex grown in Sistan climatic conditions and to evaluate its antimicrobial properties.

## 2. Materials and methods

### 2.1. Plant materials

The aerial parts of *Vitex agnus-castus* L. were collected during their flowering stage in 2021 June from the Chah Nimeh (medicinal plants collection, Institute of Agriculture, University of Zabol, Iran) (Figure 1). Collected plant samples were air-dried in shade and under room temperature conditions.

The aerial parts of vitex (leaves, stems, flowers, and seeds) were collected from the Sistan region (the medicinal plants Collection, Institute of Agricultural reaserch, Zabol, Iran), during June- July 2021 (Figure 1). Collected plant samples were air-dried under shade and room temperature conditions.



Figure 1. The aerial parts of vitex grown in Zabol climate

### 2.2. Essential oils isolation

One hundred grams of dried plants were crushed into smaller pieces, and hydrodistilled in a Clevenger-type apparatus for 4 h. The oils were stored in dark glass vials at 4 °C until the analysis time.

### 2.3. Gas Chromatography-Mass Spectrometry (GC-MS)

The compounds were analyzed on a 6890 N Agilent gas chromatograph coupled with a 5975 C Agilent mass-selective detector (Agilent Technologies, Avondale, PA, USA). A 7683 Agilent autosampler and 2  $\mu$ L of the sample were injected in splitless mode at 250°C into a 30 m  $\times$  0.25 mm  $\times$  0.5  $\mu$ m DB-5 MS capillary column and were operated by MSD Chemstation Software (Agilent Technologies). The temperature program used for the chromatographic separation was as follows: 50°C for 2 min, the temperature increase at 25°C/min to 100°C and hold for 2 min, then temperature increased at 5°C min<sup>-1</sup> to 290°C where it was finally held for 5 min. The carrier gas was helium (99.999%) and was kept at a constant flux of 1.0 ml /min. The mass spectrometer operated in electronic impact ionization mode and the energy of the electrons was kept at 70 eV. The interface was kept at 290°C. The mass spectrums were obtained at a mass ratio can range from 100 to 400 m/z to determine the appropriate mass.

### 2.4 Identification of components

The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C8–C22 n-alkanes (van den Dool and Kratz, 1963). The individual constituents were identified by their identical retention indices, referring to the known compounds taken from the literature and by comparison of their mass spectra either with the known compounds or with the Wiley mass spectral database.

### 2.5. Antimicrobial assay

Antibacterial activity of essential oils, isolated from *vitex* aerial parts was analyzed using Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests (Habbab et al., 2016). In microbiology, MIC is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation. MIC were defined as the lowest concentration of essential oil inhibiting visible growth of the bacteria (Owuama, 2017).

A collection of six organisms, including three Gram-positive and three Gram-negative bacterial strains, was used. The groups included one organism of American Type of Culture Collection (ATCC), one organism of Institute of Standards and Industrial Research of Iran (ISIRI) and four organisms of Plat Total Colony Count (PTCC). Table 1, shows the source of the bacterial strains. In this study, we used three Gram-negative bacterium, *E. coli* (PTCC 1533), *Pseudomonas aeruginosa* (ISIRI 275), *Salmonella typhi* (PTCC 1609), and three Gram-positive bacterium, *Listeria monocytogenes* (PTCC 1163), *Bacillus cereus* (ATCC 11778), and *Staphylococcus aureus* (PTCC 1112).

Serial dilutions of *vitex* essential oil were prepared in microdilution tubes with concentrations ranging between (1/2) 156.25 $\mu$ g/ml and (1/128) 20,000  $\mu$ g/ml.

Bacterial suspensions were adjusted to the logarithmic-phase growth to match the turbidity of a 0.5 McFarland standard, yielding approximately 10<sup>6</sup> CFU/mL. The same amounts of bacteria were added to all tubes and the tubes were incubated at 37 °C for 24 h.

Each tube was examined for growth and was compared with the control. Without adding bacteria, medium with no essential oil, medium containing DMSO and different essential oil concentrations were used as a control for each mentioned component, respectively. The absence of growth was defined as antibacterial activity.

Bacterial inoculum was prepared by suspension of freshly grown bacteria in sterile saline (0.85% NaCl w/v)

and was adjusted to a 0.5 McFarland standard. The MBC is the lowest concentration of antibiotics required for killing a particular bacterium. The eight dilutions were run in duplicate for the MBC test. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC by Spectro photometrical method using ELISA reader (Bio-Tek Instruments) at 580 nm.

**Table 1. The source of the bacterial strains**

Gram-positive bacterium		Gram-negative bacterium	
<i>Listeria monocytogenes</i> (PTCC 1163)	Plat Total Colony Count	<i>E. coli</i> (PTCC 1533)	Plat Total Colony Count
<i>Bacillus cereus</i> (ATCC 11778)	American Type of Culture Collection	<i>Pseudomonas aeruginosa</i> (ISIRI 275)	Institute of Standards and Industrial Research of Iran
<i>Staphylococcus aureus</i> (PTCC 1112)	Plat Total Colony Count	<i>Salmonella typhi</i> (PTCC 1609)	Plat Total Colony Count

### 3. Results and discussion

The results of identifying the active compounds in the essential oil of vitex aerial parts are reported in Table 2. In vitex essential oil, 30 bioactive compounds were identified based on retention time and mass spectra.

The most important compounds are 1,8-Cineole (39.16%), Sabinene (8.78%),  $\beta$ -Myrcene (6.44%), Sclareol (4.3%), and *trans*-Caryophyllene (3.17%). In other words, this sample is a 1,8- Cineole chemotype (Table 2).

**Table 2. Chemical composition of essential oil from flowering branch of *Vitex agnus-castus***

No	Compounds	Percentage of compounds	Retention time
1	$\alpha$ -Pinene	1.09	7.729
2	$\beta$ - Pinene	2.33	8.03
3	Sabinene	8.78	9.602
4	$\beta$ -Myrcene	6.44	9.8
5	Isoborneol	2.21	9.090
6	$\alpha$ -Terpinene	2.11	9.981
7	Limonene	1.43	10.049
8	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy	0.87	10.267
9	1-Cyclohexene-1-methanol, $\alpha,\alpha,4$ -trimethyl	1.15	10.687
10	1,8-Cineole	39.16	11.045
11	<i>trans</i> -Sabinene Hydrate	0.93	11.169
12	<i>trans</i> -Caryophyllene	3.17	11.356
13	$\beta$ -Farnesene	1.34	11.704
14	$\alpha$ -Humulene	1.17	11.922
15	$\alpha$ -Terpinyl Acetate	1.02	12.441
16	Bicyclogermacrene	1.09	12.518
17	Caryophyllene Oxide	1.93	12.57
18	Retinol Acetate	1.78	12.638
19	Spathulenol	1.97	12.679
20	<i>tau</i> -Cadinol	0.82	13.266
21	$\alpha$ -Bisabolol	2.26	13.406
22	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-2-butanone	2.34	13.541
23	2,6-Bis(aminomethyl)-2,6-adamantane diol	1.18	14.36
24	Sclareol	1.14	14.495
25	4-Bromo-1-Naphthalenamine	0.93	14.521
26	$\beta$ -n-Methylionone	1.84	14.713
27	Dihydrosclarene	2.57	14.947
28	Sclareol	4.3	15.222
29	Thunbergol	1.86	15.595
30	7-a-Hydroxymintlactone	0.79	18.086
<b>Total</b>		<b>100</b>	

Other studies have identified these compounds as the most important compounds in vitex fruit essential oil grown in Turkey (Sarikurkcu et al., 2009; Eryigit et al., 2015; Tin et al., 2017). Another study that looked at the chemical composition of essential oils over time found that the following components are the most important:  $\alpha$ -pinene (26.99%), 1,8-cineole (14.20%), *trans*-caryophyllene (9.13%), sabinene (8.29%), germacrene-B (8.20%), limonene (6.53%), 1,6,10-dodecatriene (6.37%), while main components of the essential oils obtained in fruit

maturity period were detected as 1,8-cineole (28.34%),  $\alpha$ -pinene (26.96%), sabinene (16.36%), and limonene (9.08%) (Tin et al., 2017).

A study on vitex growing in Nigeria considered the most important active compounds in its leaves were  $\beta$ -pinene (20.0%), viridifloral (9.8%),  $\alpha$ -pinene (9.1%), *cis*-o-cymene (8.4%), 1,8 cineole (6.7%) and  $\beta$ -farnesene (5.4%) (Hamid et al., 2010).

In the Southern-West Algeria region, the major components in the oil of flowers were 1,8-cineole

(17.16%), caryophyllene (12.94%) and terpinen-4-ol (10.22%), while the dominant compounds in the oil of the seeds were 1,8-cineole (14.92%), cedrelanol (13.95%) and 7 $\alpha$ -isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (13.90%) (Habbab et al., 2016).

The major components of vitex essential oil from Brazil were identified as 1,8-cineole (47.9%), terpinyl  $\alpha$ -acetate (11.6%), sabinene (11.2%) and caryophyllene oxide (9.7%) (Ricarte et al., 2020).

Changes in the active ingredients of medicinal plants vary depending on climate conditions, geographical

conditions, medicinal organs, and the method of extracting essential oils (Mazandarani et al., 2013, Yaldiz and Sekeroglu, 2013).

Previous studies have examined the antimicrobial effects of vitex fruits essential oil. Their results indicate the sensitivity of gram-positive and gram-negative bacteria to this essential oil compared with chemical antibiotics (ampicillin and ofloxacin) (Eryigit et al., 2015). The researchers reported that 1,8-cineole and  $\alpha$ -Pinene showed very high antimicrobial potency as well (Stojkovic' et al., 2011).

**Table 3. Antibacterial activity of vitex essential oil**

Bacteria	Type of bacteria	MIC ( $\mu$ g/ml)	MBC ( $\mu$ g/ml)
<i>Escherichia coli</i> (PTCC 1533)	Gram negative	5000	5000
<i>Pseudomonas aeruginosa</i> (ISIRI 275)	Gram negative	20000	20000
<i>Salmonella Typhi</i> (PTCC 1609)	Gram negative	10000	10000
<i>Listeria monocytogenes</i> (PTCC 1163)	Gram positive	625	625
<i>Bacillus cereus</i> (ATCC 11778)	Gram positive	312.5	312.5
<i>Staphylococcus aureus</i> (PTCC 1112)	Gram positive	5000	5000

MIC= Minimum Inhibitory Concentration; i.e., the lowest concentration of a particular antibiotic needed to kill bacteria.

MBC= Minimum Bactericidal concentration; i.e., the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by  $\geq$ 99.9%.

Significant antibacterial activity of essential oil was recorded against that of the examined multi-resistant pathogenic bacteria, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*.

*Bacillus cereus* (ATCC 11778) was the most sensitive strain against this essential oil (Table 3). On the other hand, in our experiment, gram-positive bacteria were more sensitive than gram-negative bacteria.

Eryigit et al., (2015) reported that, after *Enterococcus faecalis*, *Bacillus subtilis* was one of the sensitive species with a 12 mm inhibition zone. In other studies, the essential oils of vitex seeds and leaves showed antibacterial potency against *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa*, respectively.

While the oil of leaves and flowers showed high antifungal activity against *Penicillium escpansum*, *Aspergillus flavus* dominant compounds, respectively (Habbab et al., 2016).

#### 4. Conclusion

Vitex's healthy and beneficial effects should be further analysed. Considering the essential oils obtained from areal parts of vitex, it seems that 1,8-cineole and sabinene were highly present in this plant. The study revealed significant antibacterial activity of the investigated essential oils. The examined oil exhibited high resistant pathogenic bacteria, which was found to be in correlation to the content of mainly monoterpenes and sesquiterpenes. These results indicate that essential oils could be served not only as flavor agents but also as safe antiseptic supplements in preventing the deterioration of foodstuff, beverage products, and pharmaceuticals.

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## Physiological, antioxidant and yield responses of barley cultivars in nutrients-mediated alleviation of salinity stress

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Zinc sulfate

### ABSTRACT

Salinity is one of the most important abiotic stresses and variables restricting the successful production of plant products around the world, with negative consequences for plant development and other metabolic processes. The effect of nutrient management (control, 0.5 percent K<sub>2</sub>SO<sub>4</sub>, 0.5 percent ZnSO<sub>4</sub>, and 1.5 Mm salicylic acid) on physiological parameters, antioxidant activities, and grain yield responses of three barley (*Hordeum vulgare* L.) cultivars (Armaghan, Goharan, and Mehr) were examined under salinity stress (1 and 12 dS/m of salinity). Salinity stress considerably lowers growth, yield components, and grain yield, according to the findings. The number of grains per spike and 1000-grain weight of all cultivars tested increased after foliar application of salicylic acid. ZnSO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, and salicylic acid influenced grain and biological yields. At a salinity of 12 dS/m, foliar treatment of ZnSO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, and salicylic acid boosted peroxidase, superoxide dismutase, ascorbate peroxidase, and catalase while decreasing hydrogen peroxidase and malondialdehyde. Under the influence of foliar application, the relative water content increased by 12 percent, while the leaf water potential dropped by 8 percent. Salicylic acid treatment had a stronger impact on Mehr cultivar yield and physiological parameters than ZnSO<sub>4</sub> or K<sub>2</sub>SO<sub>4</sub>. These findings revealed that under the impact of salicylic acid, the Mehr cultivar was more appropriate than other cultivars.

### 1. Introduction

Barley is a major staple food crop in the world. Increasing grain yield and improving quality are of great importance to the increasing human population (Curtis and Halford, 2014). Although all abiotic stresses adversely affect barley growth and production, water scarcity imposes the most severe effects on this crop (Gonzalez et al., 2010). Researchers have stated that plants are very sensitive to salinity stress at pollination and grain-filling stages. Salinity stress leads to overproduction of Reactive Oxygen Species (ROs), which is known as a defense mechanism of plants (Thapa et al., 2018). Under salinity stress conditions, tolerant cells activate their antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which contribute to ROs accumulation (Sinclair, 2011).

Salicylic acid is a plant growth regulator that plays a significant role in abiotic stress. It was revealed that salicylic acid increased the abscisic acid content, leading to the accumulation of proline, and that soaking grains in acetyl-salicylic acid improved the salinity tolerance in wheat (Farooq et al., 2009).

Low crop productivity leads to the problem of the world's food security being under salinity stress, particularly in those areas that receive less rainfall annually (Beheshti and Behboodifard, 2010). Adequate moisture supply is vital for successful germination and crop productivity (Manivannan et al., 2008). During the spring season, high temperatures and higher rates of transpiration cause severe barely yield losses due to low moisture supply. Higher yield losses are reported when the spells of salinity stress reach their limit, especially during the critical growth

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periods (reproductive and grain filling stages) of the crop (De Jonge et al., 2015). Barely, due to its higher nutritional quality with respect to grain and fodder, it ranks 3rd among the cereal crops around the world. It is grown under a wide range of physiographic, soil, and climatic conditions. Salinity stress suppresses the crop yield by reducing the yield attributes (grain weight, grain number) (De Jonge et al., 2015).

Potassium creates resistance against salinity stress in the plant body by activating the antioxidant enzymes (Asada, 2000; Martineau et al., 2017; Hussain et al., 2018a, b). The current scenario for the causes of the low yield of barely is the decreasing availability of potassium ( $0.8 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) in Pakistan and other developing countries as compared to the world's potassium use ( $15.1 \text{ kg ha}^{-1} \text{ year}^{-1}$ ). For sustainable and more yielding growth barely under salinity stress, the presence of potassium in ample quantity in the plant tissues is very vital (Bly et al., 2002; Valadabadi and Farahani, 2009). Barely crop is also very sensitive to micronutrient deficiencies, particularly zinc deficiency. Indeed, under zinc-deficient conditions, the activities of antioxidant enzymes become limited, resulting in oxidative damage to the cell membrane of barely plants (Aref, 2010). The major yield limiting factors among the nutrients under abiotic stress include the low supply of zinc in semi-arid regions (Cakmak, 2008; Balakrishnan et al., 2017).

The goal of this study was to explore the influence of nutrient management on physiological parameters, antioxidant activities, and grain production responses of three barley cultivars under salt stress.

## 2. Materials and methods

Three barley cultivars, including (Armaghan, Goharan, and Mehr), were selected. Seeds of uniform size of three cultivars were sown in a field at the research farm of the Kabotarabad station in Isfahan, Iran, during the 2018–19 growing season. The crop was irrigated with good-quality

irrigation water and salinity water quality ( $12 \text{ dS/m}$ ).

The soil texture is loam, with an acidity of 6.8 and an electrical conductivity of  $7.62 \text{ dS/m}$ . The experiment was set up in a split-split-plot complete randomized block design with three replicates. The main plots were salinity treatments ( $1$  and  $12 \text{ dS/m}$  of NaCl), foliar application (control,  $0.5\%$   $\text{K}_2\text{SO}_4$ ,  $0.5\%$   $\text{ZnSO}_4$ , and  $1.5 \text{ Mm}$  salicylic acid), and the three barley cultivars as sub-subplots.

Measurements included the relative water content of the flag leaf (RWC) (Castillo, 1996), soluble sugars (Zhang et al., 2017) and soluble proteins (Bradford, 1976); activities of peroxidase (POD) (Cakmak et al., 1993), ascorbate peroxidase (APX) (Nakano and Asada, 1981), catalase (CAT) (Aebi, 1984), and superoxide dismutase (SOD) (Dhindsa and Matow, 1981); levels of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Veljovic-Jovanovic et al., 2002) and malondialdehyde (MDA) (Hodges et al., 1999); concentrations of calcium, potassium and magnesium by flame photometer (model 410; Corning Inc., Corning, NY, USA); and leaf water potential ( $\Psi_w$ ) (PMS Instrument Company, Albany, OR, USA). At maturity, grain yield, number of grains per spike, 1000 grain weight, and harvest index were measured.

Analysis of variance was performed on the data for each parameter by using SAS version 9.2 software (SAS Institute, Cary, NC, USA). Significant differences among mean values were compared using Duncan's multiple range test (at  $P \leq 0.05$ ).

## 3. Results and discussion

Salinity stress  $12 \text{ ds/m}$  significantly reduced the grain number per spike by 25.33% in Mehr and 34.21% in Armaghan. The negative impact of salinity stress on the number of grains per spike was alleviated by the application of potassium sulfate and zinc sulfate (Table 1). The 1000-grain weight of barley cultivars decreased significantly under salinity stress. The cultivar Mehr had a higher 1000-grain weight under salinity stress (Table 1).

**Table 1. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on yield, yield components, biological yield and harvest index of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.**

Salinity stress	Chemical treatment	No. of grains per spike			1000-grain weight (g)			Grain yield ( $\text{g m}^{-2}$ )		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	30.01b	30.56b	29.36b	52.00ab	52.30b	51.10a	455.00b	570.87b	455.87c
	Potassium	30.61b	33.12a	31.12a	52.32a	52.42b	50.22b	452.65bc	570.32b	480.32a
	Zinc	30.54b	31.32b	30.52b	52.81a	52.01b	50.81a	490.23a	482.23c	445.32d
	Salicylic acid	33.52a	33.63a	31.23a	53.15a	53.15a	51.15a	476.47d	578.32a	472.23b
12 (dS/m)	0	25.11e	20.56e	21.56e	32.52e	31.02d	31.12e	310.27g	467.77d	257.87g
	Potassium	26.13d	24.02d	22.42d	35.05cd	34.15cd	32.15d	344.25e	435.53e	315.23ef
	Zinc	27.15cd	24.12d	22.12d	35.00cd	34.80cd	32.00d	340.42f	430.30e	320.40e
	Salicylic acid	28.32c	25.12c	23.12c	36.01c	36.11c	33.01c	340.13f	405.45f	285.45f

For each parameter, means followed by the same letter are not significantly different at  $P=0.05$

The decline in 1000-grain weight was considerably less in plants supplied with potassium sulfate and zinc sulfate than when these treatments were not applied. Therefore, foliar application of these treatments can significantly improve 1000-grain weight under field salinity conditions; The maximum weight of 1000 grains under the influence of salicylic acid was obtained by the Mehr cultivar (Table 1). However, foliar application of zinc sulfate, potassium

sulfate, and salicylic acid caused a significant increase in grain yield under saline conditions. The effect of salicylic acid was greater than that of potassium sulfate or zinc sulfate applied (Table 1).

In three cultivars, the biological yield decreased significantly under salinity stress conditions; however, potassium sulfate and zinc sulfate -treated plants had higher biological yields than untreated plants under salinity stress

alone. The effect of salicylic acid application on biological yield was greater than that of potassium sulfate or zinc sulfate applied (Table 1). Salinity stress decreased the

harvest index for Goharan only. Foliar application of salicylic acid significantly promoted the harvest index of three barley cultivars under saline conditions (Table 1).

Table 1 Continued.

Salinity stress	Chemical treatment	Biological yield (g m <sup>-2</sup> )			Harvest index		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	1376.62b	1300.52b	1310.85b	33.18b	33.83b	31.03d
	Potassium	1357.10c	1310.23ab	1170.23c	34.45a	33.71b	39.1a
	Zinc	1389.23a	1315.02ab	1545.02a	33.50b	33.38b	33.08c
	Salicylic acid	1350.23d	1364.02a	1164.02c	32.38bc	32.80b	34.10b
12 (dS/m)	0	700.68g	735.32e	715.82e	25.00d	33.69b	33.09c
	Potassium	750.10f	770.50d	670.50f	25.84d	34.03ab	33.83c
	Zinc	753.14ef	770.25d	678.35f	28.02c	35.92ab	32.32cd
	Salicylic acid	768.12e	900.88c	890.88d	28.45c	38.02a	33.02c

For each parameter, means followed by the same letter are not significantly different at P=0.05

Salinity affects water relations and photosynthetic pigments of barely crops and leads to a reduction in grain and biological yield. Osmotic adjustment is a mechanism for maintaining transpiration under drought, but its role in maintaining growth and yield is not so simple (Turner, 2018). Values of LWP, OS, and TP declined with salinity stress due to insufficient water available to fulfill the needs of transpiration (Waraich et al., 2012; Wang et al., 2013). Potassium and zinc improve water relations and chlorophyll content as these nutrients are part of many biochemical and physiological processes (Kambe et al., 2015) and under stress, the concentration of potassium in the cell maintains these processes and resists the production of oxygen reactive species (Morshedi and Farahbakhsh, 2010; Osakabe et al., 2014), which leads to tolerance. As a result, because the potassium concentration in the soil was moderate, applying potassium at a lower rate than

recommended did not improve the processes, even when supplemented with zinc (Table 2). Although zinc is an important plant micronutrient, its role under salinity stress is not well defined. Plants treated with Potassium and zinc had significantly higher soluble sugar content than untreated plants under salinity stress alone.

The influence of salicylic acid on soluble sugars in plants under salinity stress tended to be greater than that of potassium or zinc applied. The responses of cultivars to potassium and zinc varied significantly, with Mehr being more responsive. In potassium, zinc, and salicylic acid treatments and under salinity stress, soluble sugar content was 19.75%, 15.20%, and 27.22% higher, respectively, in Goharan, and 12.08%, 15.88%, and 21.34% higher in Armaghan than with no foliar application (Table 2). In three cultivars, the levels of soluble proteins decreased markedly under saline conditions.

Table 2. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 mM Salicylic acid of soluble sugars, soluble proteins and mineral nutrients in the leaves of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions Measures of sugars and minerals by dry weight, protein by fresh weight.

Salinity stress	Chemical treatment	Soluble sugars			Soluble proteins			Potassium		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	33.80e	33.12f	32.12f	16.50b	15.50bc	14.50bc	58.63f	47.08ef	46.08e
	Potassium	36.12d	37.32e	36.32e	14.85c	15.32bc	13.32c	58.52f	48.10e	46.10e
	Zinc	37.10cd	37.86e	36.86e	14.00cd	15.69bc	14.69bc	62.00e	52.01d	51.01d
	Salicylic acid	37.18cd	40.00d	59.00c	15.65c	18.23a	17.23a	63.00d	52.11d	51.11d
12 (dS/m)	0	41.11c	52.02c	56.02d	10.52e	12.36d	12.36d	66.23c	65.00c	65.00c
	Potassium	48.23b	65.51ab	64.51ab	13.02d	14.36c	13.36c	71.85b	73.10b	72.10b
	Zinc	49.26b	61.45b	61.45b	13.00d	14.32c	13.32c	73.15a	78.00ab	78.00a
	Salicylic acid	33.80e	33.12f	32.12f	16.50b	15.50bc	14.50bc	58.63f	47.08ef	46.08e

For each parameter, means followed by the same letter are not significantly different at P=0.05

Table 2 Continued.

Salinity stress	Chemical treatment	Magnesium		Calcium	
		Armaghan	Mehr	Armaghan	Mehr
1 (dS/m)	0	2.30b	2.31e	2.30b	2.31e
	Potassium	2.31b	2.28e	2.31b	2.28e
	Zinc	2.30b	2.29e	2.30b	2.29e
	Salicylic acid	2.29b	2.31e	2.29b	2.31e
12 (dS/m)	0	2.88ab	5.07b	2.88ab	5.07b
	Potassium	2.85ab	5.10b	2.85ab	5.10b
	Zinc	3.02a	5.65a	3.02a	5.65a
	Salicylic acid	3.10a	5.71a	3.10a	5.71a

For each parameter, means followed by the same letter are not significantly different at P=0.05

Application of potassium and zinc improved the soluble protein levels of salinity -stressed plants of three cultivars compared with plants exposed to salinity stress without

potassium and zinc application, and the effect of salicylic acid on soluble protein content was greater than that of potassium or salicylic acid applied. Foliar application of

salicylic acid also significantly increased soluble protein content by 6.80% and 15.32%, respectively, in Armaghan and Mehr under non-stress conditions (Table 2). Salinity-stressed plants fed with potassium and zinc accumulated a greater concentration of potassium than control plants. Supplementation with zinc and salicylic acid caused a marked increase in magnesium concentration in salinity-stressed plants compared with those receiving no foliar treatment (Table 2). Calcium concentration increased significantly in three cultivars under salinity stress; foliar application of potassium, zinc, and magnesium caused a

further increase in this nutrient only in Mehr. The concentrations of the three mineral nutrients (potassium, magnesium, and Calcium) were greater in Mehr than in Armaghan under salinity stress conditions (Table 2).

In three cultivars, application of potassium, zinc, and salicylic acid significantly increased POD activity in salinity stressed plants; the influence of salicylic acid was greater than that of potassium or zinc. POD was much higher in Mehr than in Armaghan under salinity stress conditions, especially with foliar-applied salicylic acid (Table 3).

**Table 3. Influence of separate or application of 0.5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on activities of peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), as well as levels of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.**

Salinity stress	Chemical treatment	POD (U mg <sup>-1</sup> protein)			SOD (U mg <sup>-1</sup> protein)			APX (U mg <sup>-1</sup> protein)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	23.18d	37.02f	37.12e	3.20d	4.32d	5.14c	2.85c	2.18d	2.18d
	Potassium	23.46d	39.32e	37.12e	3.18d	4.35d	4.24d	2.04cd	2.22d	2.32d
	Zinc	23.62d	36.25f	34.15f	4.00c	4.07d	3.13e	2.75c	2.86c	2.86c
	Salicylic acid	24.88c	39.98e	35.98f	4.50b	5.02c	3.14e	2.77c	3.83b	2.73c
12 (dS/m)	0	28.19b	65.56d	63.56d	5.00b	7.02bc	10.16a	3.14b	3.87b	3.77b
	Potassium	29.26b	74.32b	75.32b	5.50ab	8.02b	9.13a	4.85a	4.62ab	4.62ab
	Zinc	28.09b	70.65c	69.65c	5.50ab	8.32b	6.32b	4.92a	4.88a	4.78a
	Salicylic acid	32.87a	80.36a	79.36a	6.01a	9.54a	6.42b	4.87a	4.86a	4.96a

For each parameter, means followed by the same letter are not significantly different at P=0.05

**Table 3 Continued.**

Salinity stress	Chemical treatment	CAT (U mg <sup>-1</sup> protein)			H <sub>2</sub> O <sub>2</sub> (U mg <sup>-1</sup> protein)			MDA (U mg <sup>-1</sup> protein)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	2.15e	2.12d	3.12cd	8.71a	5.23c	5.23bc	5.42e	5.54d	3.14e
	Potassium	2.16e	2.10d	3.10cd	8.02b	8.00b	5.00c	4.00f	4.14e	4.14d
	Zinc	2.15e	2.13d	3.23cd	8.00b	8.12bc	5.12c	3.47g	3.23f	3.23e
	Salicylic acid	2.57d	2.89c	3.89c	7.81b	8.00b	5.00c	3.20h	3.14f	3.14e
12 (dS/m)	0	4.11c	3.21b	4.31b	8.62a	8.71a	5.71a	15.20a	10.36a	9.56a
	Potassium	4.41b	3.84a	4.84a	8.01b	8.23b	5.23bc	14.35b	9.23b	8.15b
	Zinc	4.55b	3.02b	4.02bc	8.58a	8.34b	5.34b	11.63c	6.32c	5.42c
	Salicylic acid	4.84a	3.87a	4.87a	8.78a	8.34b	5.34b	11.23d	6.42c	5.52c

For each parameter, means followed by the same letter are not significantly different at P=0.05

Plants treated with potassium or zinc had greater SOD activity than those grown solely under water limitation. The effect of salicylic acid was greater than that of zinc applied. Varietal response to potassium and zinc varied significantly for SOD activity; Mehr was more responsive. In addition, under normal salinity conditions, application of salicylic acid significantly promoted SOD activity relative to no foliar application in both barley varieties (Table 3).

Optimal potassium-zinc nutrient supply resulted in

increased dry matter production due to increased activation of enzymes and many physio-chemical processes (Kambe et al., 2015). Soil application of zinc and potassium synergistically enhances chlorophyll production, which results in more photosynthetic assimilates (Tariq et al., 2014) and ultimately produces higher yields (Abid et al., 2016) even under salinity stress. Irrigation water use efficiency (IWUE) is a very important attribute as it describes the efficiency of total applied water.

**Table 4- Influence of separate or application of .5 % Potassium sulfate, 0.5 % Zinc sulfate, and 1.5 Mm Salicylic acid on relative water content and leaf water potential of three barley cultivars (Armaghan, Goharan and Mehr) under field salinity stress and non-stress conditions.**

Salinity stress	Chemical treatment	Relative water content (%)			Leaf water potential (-MPa)		
		Armaghan	Mehr	Goharan	Armaghan	Mehr	Goharan
1 (dS/m)	0	72.12a	73.11b	70.01b	1.73e	1.80c	1.60c
	Potassium	72.31a	74.19a	73.09a	1.98d	1.78c	1.61c
	Zinc	72.00a	74.20a	73.10a	1.35fg	1.78c	1.63c
	Salicylic acid	72.10a	74.10a	73.00a	1.53f	1.68d	1.78bc
12 (dS/m)	0	60.23d	67.02d	65.42e	2.69a	2.97a	1.97a
	Potassium	65.55c	68.32c	67.32d	2.40b	1.96b	1.87b
	Zinc	65.20c	69.23c	66.33e	2.31b	1.43d	1.63c
	Salicylic acid	69.60b	68.20c	66.25e	2.11c	1.75cd	1.85b

For each parameter, means followed by the same letter are not significantly different at P=0.05

The IWUE in the current study was higher under SD than under MD and WW, which indicates barely used water

more efficiently when applied in limited amounts. Under WW conditions, IWUE was low due to more wastage of

irrigation water. Ul-Allah et al. (2014,15) reported higher IWUEs of fodder crops, including barely under water stress conditions in a semi-arid climate and suggested reevaluating the irrigation scheduling for optimum water use efficiency. As application of zinc combined with higher levels of potassium produces maximum biological yield, therefore, IWUE was also maximum for this treatment (Table 4). Higher IWUEs with the application of potassium-zinc nutrition were due to the role of these nutrients in osmotic adjustment and regulation of biochemical processes under water stress conditions (Osakabe et al., 2014; Kambe et al., 2015).

The Activity of APX also increased in both barley varieties under salinity stress, and this increase was more pronounced in Goharan. Application of potassium sulfate and zinc sulfate had no significant effect on APX activity in Armaghan under either salinity regime, whereas in Goharan, APX significantly increased with the application of zinc and salicylic acid under normal salinity conditions and with the application of potassium, zinc and salicylic acid under salinity stress (Table 3).

Levels of H<sub>2</sub>O<sub>2</sub> increased markedly under saline conditions. Plants treated with potassium and zinc had lower H<sub>2</sub>O<sub>2</sub> levels than plants under salinity stress alone. Furthermore, the influence of salicylic acid application on H<sub>2</sub>O<sub>2</sub> content was greater than with either potassium or zinc applied. With application of potassium, zinc, and salicylic acid, and under salinity stress, H<sub>2</sub>O<sub>2</sub> content was lower than with no foliar application in both cultivars (Table 3).

For any food and feed crop, nutritional quality has the same importance as that of yield. Grain starch, oil and protein content are important for human consumption. The Reduction in gain nutritional content under salinity stress has been attributed to the downregulation of enzymatic activities (Halford et al., 2014; Ignjatovic-Micic et al., 2015; Kambe et al., 2015), which results in lower conversion to assimilate into grain starch, oil, and proteins. Plant researchers believe that because of the effect of water stress on nutritional quality in a climate change scenario, potassium-zinc improves regulation of enzymatic activities under salinity stress, which results in improvement of these attributes (Valadabadi and Farahani, 2009).

## Conclusion

Salinity stress significantly reduces growth, yield components, and grain yield in barley. Foliar salicylic acid reduces the negative effects of salinity stress and, consequently, improves. The results of the study highlight the role of Potassium sulfate and zinc sulfate applications in regulating the salinity stress response of barley, suggesting that salicylic acid is involved in physiological activities.

These results showed positive effects of potassium and salicylic acid in terms of increased antioxidant activity as well as relative water content and leaf water potential. In addition, potassium sulfate and zinc sulfate stimulated the active accumulation of some The effects of salicylic acid application on the Mehr cultivar on yield and physiological parameters were greater than those of potassium sulfate or

zinc sulfate. Therefore, the Mehr cultivar was more suitable than other cultivars under the influence of salicylic acid.

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# Guide for Authors

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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.

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