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ORIGINAL ARTICLE

EFFECT OF NITROGEN SOURCES AND ZEOLITE APPLICATIONS ON THE NITRATE CONTENT AND NITRATE REDUCTASE ACTIVITY OF LETTUCE (*LACTUCA SATIVA* L.) LEAVES

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Abstract: Leafy vegetables can accumulate harmful nitrate more than others. A study conducted in the field of Al-Najaf Agriculture Directorate to evaluate the effect of the sources of nitrogen and zeolite on the nitrate content and nitrate reductase activity of the lettuce plant. The first factor was the source of nitrogen including urea, liquid organic and biological fertilizers, as well as the control treatment. The second factor was three rates of zeolite including 0, 10, and 20 g. plant^{-1}. All applications were added to the plant rhizosphere except the liquid organic nitrogen was applied on the leaves. A factorial experiment using RCBD with three replicates was adopted in this study. Duncan's test was used to compare the treatment means at a probability level of 0.05. The results show that nitrate reductase activity in the outmost leaves was significantly influenced by urea fertilizer to reach 57.14 IU over control treatment 15.1 IU. Further, the activity was reduced by the application of zeolite the rate of 20 g. plant^{-1} which reached 49.58 IU compared with control treatments 75.11 IU. The results also show that the sole applications of biological nitrogen fertilization and zeolite application at the rate of 20 g. plant^{-1} can reduce the nitrate content in the plant parts including stem (389.6 and 331.5 mg.Kg^{-1} d.wt), outmost leaves (193.9 and 54.8 mg.Kg^{-1} d.wt), middle leaves (135.3and 101.8 mg.Kg^{-1} d.wt), and inner leaves (89.6 and 60.6 mg.Kg^{-1} d.wt), respectively compared with control treatments. In addition, the interaction between biological nitrogen fertilizer and zeolite application at the rate of 20 g. plant^{-1} reduced the nitrate content to the minimum. The study concluded that nitrate content reached the maximum in the stem of the lettuce. In addition, the outmost leaves recorded higher nitrate than the inner leaves, while the middle leaves recorded moderated nitrate content.

***Key words*:** Lettuce, Urea, Organic fertilizers, Bio-fertilizers, Zeolite, Nitrate content, Nitrate reductase activities.

Cite this article

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1. Introduction

The increased interest in lettuce *Lactuca sativa* L., as it is one of the most used vegetables in the salad, led to an increase in the studies concerned with this important crop. Among the various factors responsible for the high yield per cultivated area, provision of nutrients and moisture plays a vital role in the productivity and quality of lettuce [Ouzounidou *et al.* (2013)]. A positive relation was known between the amount of nitrogen applied to the plant and its productivity. Therefore, farmers tend to apply a high amount of nitrogen fertilizer weekly in order to receive a high yield [Liu *et al.* (2014)]. The amount of nitrogen required to produce an adequate harvest of leafy vegetable crops is relatively high, as it increases the economic yield [Fageria (2014)]. Nitrogen also has a vital role in synthesizing proteins, nucleic acids, and chlorophyll. However, excessive use of nitrogen fertilizer cannot only raise the economic cost but also leads to physiological disruption of the plant and harm the environment including soil and water pollution in addition to its negative effect on human health.

Many results on human bodies found that consuming food, as in leafy vegetables, with a high

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content of nitrates may cause methemoglobinemia [Greer and Shannon (2005)]. Nitrates is also associated with cancer incidence and brain tumors [Ward (2009)]. Nitrate is not toxic by itself, however, the negative effect comes from the conversion of nitrates to nitrite during consumption by the digestive system [Anjana and Iqbal (2007)].

Managing nitrate content in leafy vegetable crops can be the key factor to eliminate the downside of consuming lettuce. The source of nitrogen fertilizer, the way of applying a particular type of fertilizer, and the way of the absorption that fertilizer by the plant can affect the nitrate concentration in the plant [Liu *et al.* (2014)]. The excessive use of nitrogen fertilizer on a leafy vegetable plant leads to the occurring of nitrate accumulation since the plant cannot convert all that amount of nitrate to other forms in a short time [Chen *et al.* (2004)]. Thus, adding zeolite may help to reduce the accumulation of nitrate in the plant tissue. This mineral has a selective bonding towards nitrogen forms, and other elements, because of its negatively charged molecule [Bernardi *et al.* (2015)]. Zeolite functions as a magnet to store nitrogen and release it when nitrogen depletion occurs in the rhizosphere [Mani and Mondal (2016)]. This method not only may help the plant tissue to convert the nitrate efficiently, but it may keep the nitrogen content adequate in the plant tissue [Kanjana (2017)].

The objective of this research is to study the effect of different sources of nitrogen fertilizer on the production and quantitative characteristics of lettuce as well as examine the effect of using different levels of zeolite on lettuce productivity and quantity.

2. Materials and Methods

2.1 Field preparation

The experiment was conducted during the winter growing season of 2018-2019 in a field belonged to Al-Najaf Agricultural Directorate. A sandy loam soil was prepared for the study by plowing it twice and then left to be sterilized by sunlight (Solarization) for one month. The field area was 510 m^2 divided into six equal length terraces (65 m). The drip irrigation system was used in this study, and the plants were watered as needed. Space between plants was 20 cm, and between terraces was 90 cm. Manual weeding was approached in the field without adding any herbicides substances. Ten plants were included in each experimental unit (Table 1).

Table 1: The physical and chemical properties of the field soil.

Particles Type	Unit	Amount
Clay	%	16.2
Silt	%	25.4
Sand	%	58.4
	Soil Texture	Sandy Loam
Chemical Character	Unit	Amount
EC	Decisions. miter^{-1}	3.1
pH	————	7.9
Available Nitrogen	mg. kg^{-1}	39.6
Available Phosphorus	mg. kg^{-1}	8.2
Available Potassium	mg. kg^{-1}	237
Organic Matter	%	1

In this study, a Romaine lettuce seed of Fajer variety was used. The seeds were planted in starting plug flats with 120 cells and places in the nursery. The flats were exposed to direct sunlight. The date of the seeding was September 15, 2018. The seedling that emerginated stayed in the nursery for 4 weeks, and then transferred to the permanent field.

2.2 The studied factors and statistical analysis

The research included three factors. Different sources of nitrogen were the first factor including urea ($CO(NH_2)_2$ 46% nitrogen) (10 g. Plant^{-1}) as synthetic fertilizer, *Azotobacter chroococcum* (10 g. Plant^{-1}) as bio-fertilizer, liquid organic nitrogen (3 ml. liter^{-1}), and control treatment. Synthetic and biological nitrogen fertilizer sources were added to the plant rhizosphere while the organic source of nitrogen fertilizer was applied as a foliar application. The second factor applied to the soil was three levels of Zeolite including 0, 10, and 20 g per plant. Zeolite was added close to the plant rhizosphere. Nitrogen and zeolite were applied to the plants after two weeks from the transplanting.

Bacteria Isolates (*Azotobacter chroococcum*) were identified and prepared in broth by the laboratories of the Al-Najaf Agricultural Directorate. Peat moss was sterilized by the autoclave before the bacterial inoculation was loaded on the peat moss. Finally, the Peat moss was added to the plant Rhizosphere as a bio-fertilizer. The Plate Count Technique was used to calculate the bacterial Colony Formation Unit **(**CFU) which was 1.5 ml × 10^9. A factorial experiment using Randomized Complete Block Design (RCBD) with three replicates was adopted in this study. All collected data were analyzed using the ANOVA procedure in Genstat statistical package to assess if significant differences occurred among the experimental treatments at $P < 0.05$. Duncan's test was used to separate the treatment means.

2.3 Tested parameters

Inner, mid, and outmost leaves were randomly selected from each experimental unit to determine nitrate content in the lettuce head. Nitrate content was measured for dry samples according to Cataldo *et al.* (1975) procedure. Nitrate reductase in the outmost leaves was determined based on Redinbaugh and Campbell (1985) method.

3. Results and Discussion

3.1 Nitrate content in the leaves

Data presented in Fig. 1 show that type of nitrogen fertilizer has a strong influence on nitrate accumulation in the outermost, middle, and inner leaves of lettuce. The lowest nitrate content was recorded in the biological source of nitrogen fertilization which scored 193.9, 135.2, and 89.6 mg. Kg^{-1} d.wt, while the highest nitrate content was recorded in the control treatment 207.6, 158.8 and 102.9 mg.Kg^{-1} d.wt, respectively. The rates of zeolite application affected the nitrate content in outermost, middle, and inner leaves. The rate of 20 g. $plant^{-1}$ zeolites recorded the lowest nitrate content which scored 54.8, 101.8, and 60.6 mg. Kg^{-1} d.wt, respectively. The highest content of nitrate recorded with control treatment which scored 346.4, 198.0, and 129.4 mg. Kg^{-1} d.wt, respectively.

The interaction between nitrogen and zeolite (Table 2) showed significant differences among them regarding the nitrate content in the outermost, middle, and inner leaves. The lowest was recorded with N3Z2 which was 48.7, 91.9, and 53.3 mg. Kg^{-1} d.wt, respectively. While the maximum nitrate content was obtained at the N0Z0 interaction which was 355.5, 222.2, and 137.8 mg.Kg^{-1} d.wt, respectively.

Plants supplied with biological fertilizers have low nitrate content, compared with minerally fertilized [Raupp (1996)]. The effect of biological fertilization with *Azorobacter chroocococum* on the nitrate content in the leaves may be attributed to providing the nutritional needs of the plant from the nitrogen element through its role in fixing the bio-atmospheric nitrogen in the soil. This bio-stabilized nitrogen is available by the bacteria in the form of ammonium, which assimilated immediately after its absorption by the plant into a simple amino acid.

Another significant finding can be noted from the Fig. 1. The nitrate level differed according to the position of the leaves in the lettuce head. Nitrate accumulated in the stem was the highest compared with other tested parts of the plant. In addition, the outmost leaves tend to accumulate higher levels of nitrate than the ones in the middle which in its part tend to have higher nitrate content than the inner leaves. This could be attributed to the position of the chloroplast in which nitrogen assimilates to amino acids. Chloroplast needs sunlight in order to have the required energy to reduce nitrogen. Thus, the number of formed plastids is the highest in outmost leaves, and the number dwindles in the leaves as the position of leaves gets inside the head of lettuce. Plant transfers nitrogen to these reduction places for assimilation. However, the over needed nitrogen will be stored in the vacuoles. Therefore, the closest the leaf from the core of the head is the less nitrate can be found in the leaves.

3.2 Nitrate reductase activity

Fig. 1 displays that the source of nitrogen fertilizer had affected the nitrate reductase activity. Urea and organic fertilizer resulted in significantly the highest activity of the enzyme 57.14 and 54.70 IU, respectively. However, no significant differences were observed between the urea and organic sources of nitrogen regarding the same parameter. On the other hand, the control treatment scored significantly the lowest activity of the enzyme 51.01 IU, even though it had statistically similar activity as biofertilizer 51.40 IU. Regarding zeolite treatment, the control treatment had the highest activity of nitrate reductase of 57.11 IU compared with plants treated with zeolite rate 20 g. $plant^{-1}$ which scored the lowest nitrate reductase activity of 49.58 IU. Plants treated with zeolite rate 10 g. $plant^{-1}$ were different than zeolite rate 20 g. $plant^{-1}$ and control treatment, therefore, this rate of zeolite showed moderate effect. Nitrogen and zeolite interaction showed that N2Z1 scored 60.56 IU which was the maximum activity of nitrate reductase over N0Z2 which scored the lowest activity of 42.11 IU.

This results could be related to the high level of nitrate in the plant tissue which induce nitrate reductase activity. While the low nitrate content in the tissue reduce

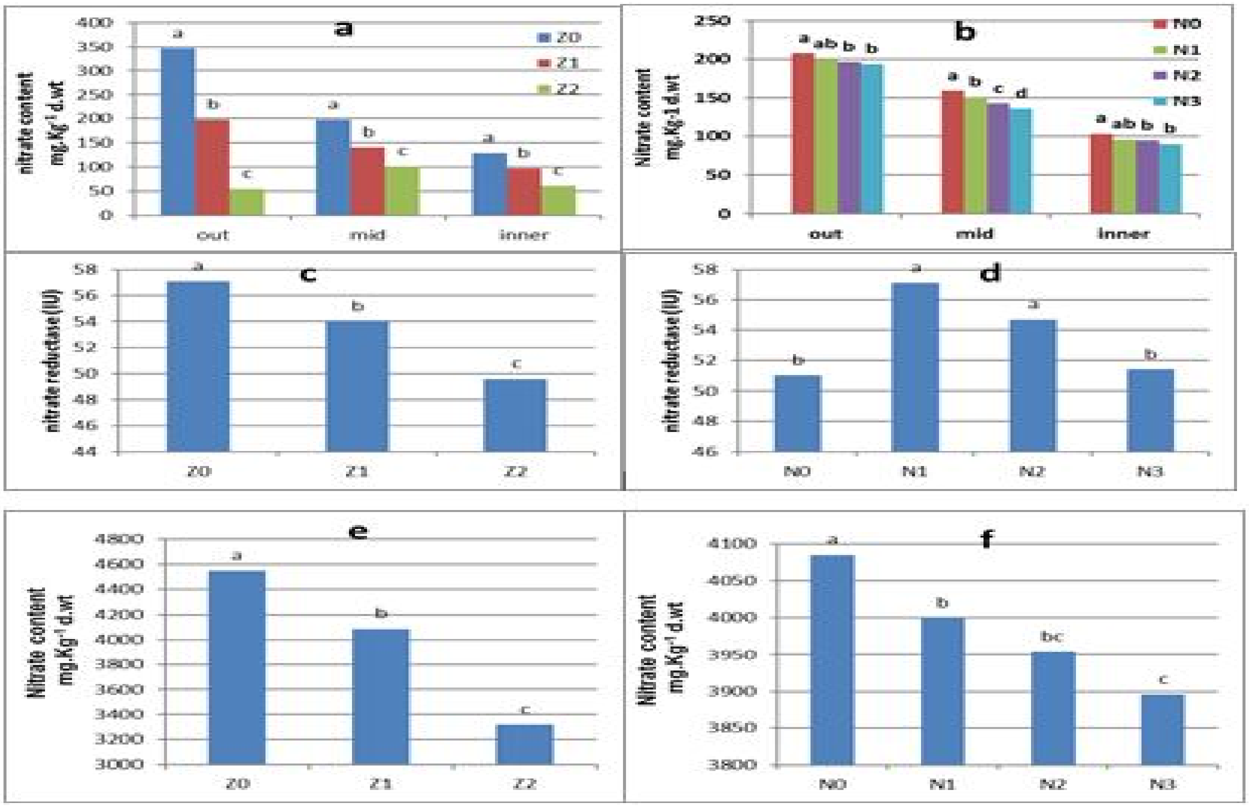

Fig. 1: a) The effect of zeolite on the nitrate content in the outmost, middle, inner leaves b) The effect of nitrogen on the nitrate content in outmost, middle, inner leaves c) The effect of zeolite on the nitrate reductase of outmost leaves d) The effect of nitrogen on the nitrate reductase of outmost leaves e) The effect of zeolite on the nitrate reductase of the stem. f) The effect of nitrogen on the nitrate reductase of the stem

Table 2: The interactions between nitrogen source and zeolite on nitrate content (mg.Kg^{-1} d.wt).

Treat	Out	Mid	Inner	Stem	Reductase in the outmost leaves
$N_0 Z_0$	355.5 a	222.2 a	137.8 a	4622 a	60.47 a
$N_1 Z_0$	347.5 a	203.7 b	128.9 a	4578 ab	58.21 ab
$N_2 Z_0$	342.6 a	188.2 c	126.7 a	4522 ab	51.24 cde
$N_3 Z_0$	340.1 a	178 d	124.4 a	4467 b	58.51 ab
$N_0 Z_1$	205 b	144.8 e	105.6 b	4156 c	50.47 cde
$N_1 Z_1$	197.6 b	143.1 ef	96.7 bc	4111 c	58.06 ab
$N_2 Z_1$	195.6 b	139.3 fg	94.4 bc	4067 c	60.56 a
$N_3 Z_1$	193 b	135.8 g	91.1 c	4022 c	46.93 e
$N_0 Z_2$	62.4 c	109.3 h	65.6 d	3478 d	42.11 f
$N_1 Z_2$	55.8 c	105 hi	62.2 d	3311 e	55.14 bc
$N_2 Z_2$	52.3 c	100.1 i	61.1 d	3272 e	52.31 cd
$N_3 Z_2$	48.7 c	91.9 j	53.3 d	3200 e	48.74 de

the activity of nitrate reductase (Table 2).

Data presented in Fig. 1 show that type of nitrogen fertilizer has influenced nitrate accumulation in the stem of lettuce. The lowest nitrate content was recorded in the biological source of nitrogen fertilization which scored 3896 mg.Kg^{-1} d.wt, while the highest nitrate content was recorded in the control treatment (4085) mg.Kg^{-1} d.wt. As for the zeolite application, the rate of 20 g.plant^{-1} recorded the lowest nitrate content which scored 3315 mg.Kg^{-1} d.wt, while the highest content of nitrate recorded with control treatment which scored 4547 mg.Kg^{-1} d.wt. The interaction between the biological source of nitrogen and 20 g.plant^{-1} zeolite application recorded the lowest value of 3200 mg.Kg^{-1}

d.wt (Table 2). The great difference between the nitrate content present in the leaves compared with the nitrate level in the stem may be related to the fact that the lettuce stem is the storage part of the plant. The plant moves the nitrate from the leaves to the stem during the metabolism waiting for its turn for the conversion. Although the nitrate content in the stem was reduced by the tested factors, the nitrate still higher than the maximum levels of nitrate that were permitted by the World Health Organization.

4. Conclusion

Bio-fertilizer, liquid organic nitrogen reduced the nitrate content and nitrate reductase activity in the leaves. Zeolite application at the application rate of 20 g.plant^{-1} decreased the nitrate content in the leaves of the lettuce plant as well as the nitrate reductase activity. The results showed that the maximum nitrate content was accumulated in the lettuce stem. Then, the outmost leaves tend to accumulate nitrate more than the inner leaves while the middle leaves had a moderated nitrate level. Therefore, it is recommended to avoid consuming lettuce stems since it may cause negative effects on human health.

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