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RESEARCH ARTICLE

USE OF PLANT BENEFICIAL MICROORGANISMS TO IMPROVE PLANT NUTRITION AND YIELD IN HEAD SALAD CULTIVATION

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ABSTRACT

In this study, commercially available four different bio-fertilizers have been used. 1) Cocktail mychorrhiza, which contained 9 species of mychorrhiza; *Glomus aggregatum*, *Glomus clarum, Glomus deserticola, Glomus etunicatum, Glomus intraradices, Glomus mosseae, Glomus monosporus, Glomus brasilianum, Gigaspora margarita,* 2) Bacteria (Seranade) which contained *Basillus subtilis*, 3) Bacteria (Roa Natura) which contained *Arthrobactersp*, 4) Microalgae (Allgrow) which contained *Chlorella spp*., and chemical fertilizers used in conventional farming were applied to the control plants. Plants were grown in pots in a controlled medium to avoid contamination. The lettuce plants grown under different treatments were compared at the end of the study; the yield, head circumference, diameter and height dimensions of the product, fresh and dry weights, chlorophyll content and the macro-and micro-nutrient element contents were investigated. As a result, with the use of bio-fertilizers in the plants, they benefited more from the nutrient elements efficiently. The addition of beneficial microorganisms to the soil in greenhouse cultivation affected the yield and quality positively.

INTRODUCTION

For thousands of years, plants have maintained their existence without the aid of any extra input so far. However, today, apart from the naturally grown plants, specific care is compulsory for the culture cultivation. It is nearly impossible to cultivate without irrigation, fertilizing and agricultural spraying. Most of the natural plants under cultivation have their own defense mechanisms developed in order to survive. Nearly all of the plants in the nature have developed a symbiotic relationship with some of the micro-organisms (like mycorrhiza and rhizobium bacteria) living on their roots and in a sense continue to co-habit. Reflecting this self-evolving co-habitation onto the plants under cultivation has enabled success on the resolution of various agricultural and environmental issues. Due to being an alternative solution to chemical fertilizers and pesticides, use of micro-organisms in organic agriculture has spread out extensively. These types of micro-organisms that increase the soil productivity and support plant cultivation are called "bio-fertilizers" and used accordingly. Injecting beneficial micro-organisms into the soil as microbial fertilizers enhances product yield and quality (Vessey, 2003). Today, intensive use of chemical fertilizers, over cultivation and irrigation of soil can clearly be noticed.

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The increase in the amount of chemical input transferred into the soil year by year has led to infertility and drought, resulting in a poor soil quality. Microbial fertilizers are important to environment-friendly sustainable agricultural practices (Bloemberg *et al.,* 2000). Bio-fertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms (Goel *et al.,* 1999). Among the bio-fertilizers that are beneficial to the crop production are *Azotobacterspp*, *Azosirilliumspp*, blue green algae, *Azollaspp*, P-solubilizing microorganisms, mycorrhizae and *Sinorhizobiumspp* (Hegde *et al.,* 1999).Other complementary nursing measures applied for the soil and the plants (such as crop alteration, disease and pesticide monitoring, plant nutrition) are observed to become more effective when used along with the beneficial micro-organisms. The most important function of the beneficial micro-organisms is to maximize the effect of plant nutrients. Mycorrhiza fungus, for example, increases the efficiency of some mineral nutrients (phosphorus to be highlighted at first) in soil. AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses (Ikiz, 2003; Ikiz *et al.,* 2009; Clark and Zeto, 2000; Turnau and Haselwandter, 2002). Nitrogen fixing bacteria link the nitrogen in the air into the soil.As a result of environmental pollution triggered by agriculture and the reflection of this to the cultivated products; organic way has stepped ahead in healthier and more environmental organic cultivation, and natural micro-organism use in vegetation has become important to for the plant to consume soil nutrients more efficiently (Badgley and Perfecto, 2007).Salad and lettuce can be cultivated all year long under open and covered conditions and can be consumed as fresh vegetables. Due to the vitamins and minerals, they are also considered to be within the appetizer vegetable group. Salad and lettuce cultivation previously carried out on open fields has also been started at greenhouses and low plastic tunnels in order to take the advantage of the higher crop prices of the winter season. With their pretty short cultivation period of 2-3 months, local salad and lettuce cultivation usually becomes the second or third product, just after or before the main vegetable cultivation. In reference to the vegetable price levels within the recent years, salad and lettuce are considered to be in the highest revenue generating group.The objective of our study is to review the yield and the quality properties affected by the compounds with cultivation supported by micro-organisms which are used in organic head salad cultivation.

MATERIALS AND METHODS

Materials

Plant Material: Robinson F_1 cultivar of head salad (Nikerson Zwaan) has been used for the research as plant material.

Bio-fertilizers used for the head salad cultivation

- Endomycorrhiza (VAM) *Glomus* types are used as cocktails (contained 9 species of mychorrhiza; *Glomus aggregatum, Glomus clarum, Glomus deserticola, Glomus etunicatum, Glomus intraradices, Glomus mosseae, Glomus monosporus, Glomus brasilianum, Gigaspora margarita*). Endo–Roots (Novozymes – Biologicals)
- Bacteria (1) - *Bacillus subtilis* QST 713 breed (min.1×10 cfu/ml). Serenade (Agraquest de Mexica) (licensed for the organic cultivation). Also being used as a bio-fungicide.
- Algae- *Chlorella spp*. Allgrow (Biotech Industri, AB V. Sweden) (licensed for the organic cultivation) Along with live cells of micro-algae, it contains all the necessary micro elements for the plant.
- Bacteria (2) content; *Arthrobacter sp*. (1×10 cfu/ ml) (licensed for the organic cultivation). Roa Natura (ROA Biotechnology - Turkey)
- Chemical fertilizer: The doses of the chemical fertilizers were applied by the producers in conventional cultivation.
- Control: No fertilizers were added to the substrate.

Method

The research was carried out as pot experiment in a plastic greenhouse; Pots of 2 liter have been filled with soil. On September 22nd was transplanted as one seedling per pot. The experiment was designed on a 3-repeat and 10-plant per repeat basis.Bio-fertilizers were applied after a recalculation on the package doses.Chemical fertilization was calculated for the lettuce as (Günay, 2005). Consequently; 40 kg/da pure N, 30kg/da pure P_2O_5, and 70 kg/da K_2O, 40kg/da CaO was used. The first dose of fertilizers was applied to the pots before the plant came out and seedling was performed on September 22nd. During the seedling, only mycorrhiza inoculation was completed, other micro-organisms were applied a few days after the seedling. The second application of the fertilizers was realized on November 8th. All of the micro-organisms were applied in total compliance with the method and the amount instructed by the related companies.Height, diameter and circumference measurements for the head salad crop were made in order to specify the properties for the yield, dry weight and the quality. It is also targeted to identify the effects of the micro-organisms on the cultivation by analyzing mineral nutrition elements (N, K, Mg, Ca, Fe, Zn, Cu and Mn) on the leaves of the lettuce. Also additional analysis was carried out to identify the level of the nitrate accumulation, a critical factor in vegetable leaves for human health. In the leaves, analyses were made for nitrogen (N), phosphor (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), mangan (Mn), copper (Cu) and zinc (Zn). The nitrogen was used as described by "Khjeldal", the phosphor was used according to the "Barton" methods (Jones et. al, 1991) and the other elements were used according to the Dry Incinerating Method in the granulated form (Anonymous, 1976; Jones, 1972), and were filtered with diluted HCI and the element readings were performed in atomic absorption spectro-photometer. The nitrate concentration in the leaves of head salad was defined via the nitration of salicylic acid in the colorimetric style (Cataldo *et al.,* 1975).

RESULTS AND DISCUSSION

Yield

After being cropped from each pot, head salads were individually weighted and the average weight values were calculated, leading to the final value of yield per decare. As depicted in the graph below, Mycorrhiza has got the lowest yield value after the control. The highest yield values were achieved in Bacteria (1), Algae, Bacteria (2), Chemical fertilizer, Mycorrhiza, respectively; the lowest yield value was achieved, as expected, through the control application for which no fertilizers were used.The present data are in agreement with those reported by (Talaat, 1995; Hanafy Ahmed *et al.,* 2000). These results are in accordance with those obtained by (Goel *et al.,* 1999) who reported that the inoculation with certain plant growth-promoting rhizobacteria may enhance crop production either by making the other nutrients available or protecting the plant from pathogenic microorganism (allelopathic effects). (Zodape, 2001) also concluded that, the increased yield productivity with bio-fertilizer application is due to microelement and plant growth regulator contained in the fertilizer. (Faheed and El-Fattah, 2008) reported that dry microalgae increase yield in lettuce. (Pavlou *et al.,* 2007) found out similar results in lettuce.

Plant Growth Parameters

Application measurements achieved are listed inTable 1.After a careful review of the data, it can be concluded that only the diameter and the fresh weight values are meaningful from the statistical point of view. The highest diameter value was measured in Algae and found to be very different from the rest. Then came the Mycorrhiza apps; the smallest diameter was measured in control and the rest being dispersed in between.

Table 1. Effects of different bio-fertilizers on plant growth parameters of head salad

Applications	Head Circumference (cm)	Diameter (cm)	Height (cm)	Dry Weight (g)	Fresh Weight (g)
Bacteria (2)	37.43	10.51 bc	13.87	15.04	124,46 ab
Bacteria (1)	36.40	9.52 bc	14.35	14.16	145,35 a
Algae	35.80	17.33 a	12.90	14.37	136,34 ab
Endomycorrhiza	35.47	11.94 b	12.40	12.64	95,08 ab
Chemical Fertilizer	36.31	9.91 bc	12.53	13.35	116,55 ab
Control	35.35	7.11 c	11.69	12.63	88,16 b
LSD %5	ns	4.21	ns	ns	49,41

Table 2. Effects of different bio-fertilizers on nutrient element contents in leaves of head salad

Applications	N(%)	K(%)	Mg(%)	Ca(%)	Cu (ppm)	Mn (ppm)	Fe(ppm)	Zn(ppm)
Bacteria (2)	1.91	4.00 a	1.27 ab	1.009	4.03	83.12	225.33 ab	5.84 b
Bacteria (1)	0.83	1.20 b	1.42 a	1.175	3.29	51.11	202.64 a-c	6.00 b
Algae	1.93	4.00 a	1.13 ab	1.342	4.89	63.88	282.50 a	10.85 a
Endomycorrhiza	1.00	2.41 b	1.25 ab	1.039	3.41	36.16	145.89 bc	4.11 b
Chemical Fertilizer	0.99	2.81 b	1.13 ab	1.357	2.98	48.79	153.50 bc	12.36 a
Control	0.65	2.14 b	0.92 b	0.694	2.79	33.79	119.63 c	5.32 b
LSD %5	ns	0.867	0.41	ns	ns	ns	83.21	4.27

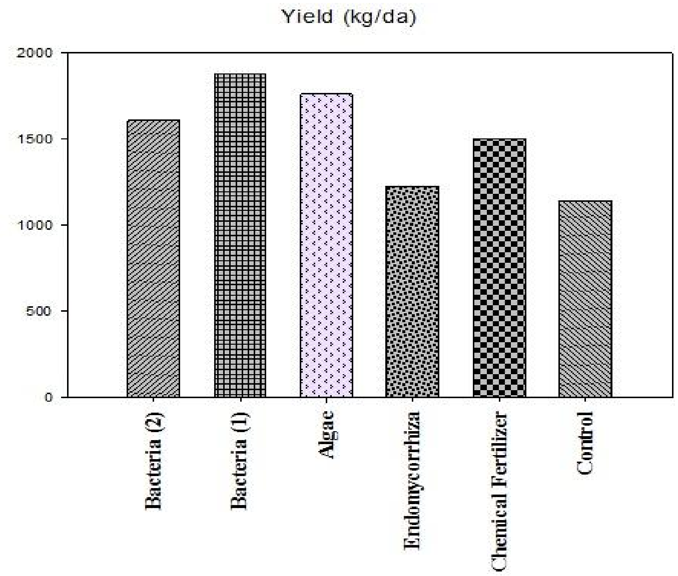

Figure 1. Effects of different bio-fertilizers on yield of head salad

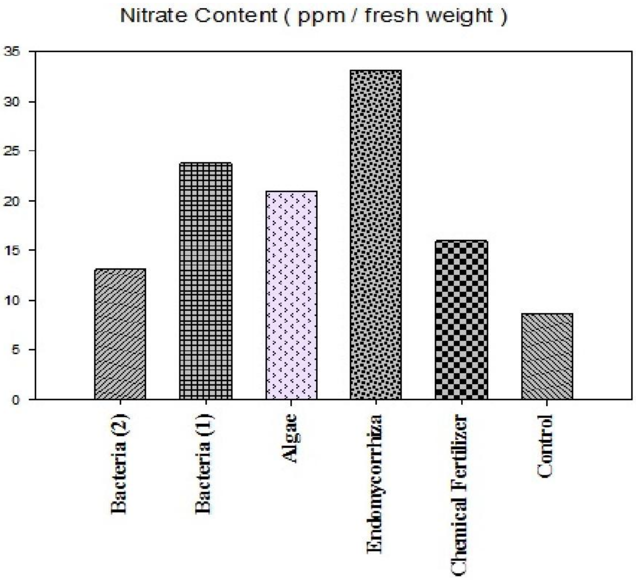

Fig. 2.Effects of different bio-fertilizers on the nitrate content accumulated on the leaves of the head salad

Fresh weight values show parallelism with the yield data and the highest value was achieved in Bacteria (1), with the control data being specifically deviated from the rest. Then came the data from Algae, Bacteria (2), Chemical fertilizer and Mycorrhiza being categorized in between. Control, on the other hand, resulted in the smallest value as expected. In referral to the dry weight values, Bacteria (2) came out with the largest value. Control, again, came up with the smallest value, though Mycorrhiza had also a very small value as a micro-organism application.Increase in fresh and dry weight resulted from the improved nutrient status of the plant due to the presence of bio fertilizers especially algae and mycorrhiza in the soil. The stimulatory effects of bio fertilizers on some growth parameters of lettuce are in accordance with the results obtained by (Al-Gosaibi, 1994; Shaaban and Mobarak, 2000; Faheed and El-Fattah, 2008; Azcon *et al.,* 2003; Jackson *et al.,* 2002; Mahmoud and Amara, 2000). Similar results were reported to affect early plant and root development, plant and root dry weight, grain yield and the N-uptake efficiency of plants (Dobbelaere *et al.,* 2002)

When the effect of the micro-organisms on the nutrient intake is studied, it can clearly be seen that there are significant differences among the applications and the so-called difference is statistically meaningful for the contents of potassium, magnesium, iron and zinc. In general; the effect of Algae, Bacteria (2) and Bacteria (1) has a positive impact on the nutrient intake. But the values of Mycorrhiza seem to be low. This result has confirmed the hypothesis that mycorrhiza act more effectively in poor nutrient soil and stress substrate. According to (Azconet al., 2003) in the medium containing low level of P (0.1 mM), the increasing N supply did not change the positive effect of AM colonization on plant macro and micronutrient uptake. High levels of P (0.5 mM) and N (9 mM) led to significantly increased nutrient contents in non-AM plants. In mycorrhiza trials, low-level fertilization is needed in comparison with the exact fertilizer amount the plant needs. To settle a new micro-organism trial with an estimated low-level fertilization process has to be targeted in order to make savings on the fertilizer amount used and keep a more environment-friendly approach.

The largest nitrate content value (33,14 ppm) was measured in the head salads cultivated with the Mycorrhiza bio-fertilizer. Then comes Bacteria (1) and Algae bio-fertilizers with 23,79 ppm and 21,00 ppm respectively. Bacteria (2) bio-fertilizer with a 13,11 ppm value has even a lower nitrate content in comparison to the Chemical fertilizers (15,96 ppm). As expected, the control application without any fertilizers reflected a nitrate value of 8,67 ppm as the lowest within the group.(Hanafy-Ahmed *et al.,* 2000) reported that bio fertilizer applications increased some nutrient element concentrations in head salad; however, also decreased nitrate accumulation.

According to (Hanafy-Ahmed *et al.,* 2000) simple organic molecules such as sugars, free amino acids and total soluble phenols may act as an osmoticum for the regulation of plant osmosis. These simple organic molecules or solutes can replace nitrate in the cell vacuoles. In the present work, there was a negative correlation between the nitrate accumulation and the organic compounds of sugars, free amino acids and total soluble phenols. In our study, only the Algae, which included *Arthrobacter spp.*, decreased the nitrate content; and (Hanafy-Ahmed *et al.,* 2000; Blom-Zandstraet al. 1988) found similar results. Other bio fertilizers did not suppress especially the mycorrhiza nitrate accumulation; however, an accumulation that is harmful for human health did not occur, the level was much below the harmful level.

The nitrate content values found within the head salad lettuce leaves cultivated with different applications remain fairly below the value of 805 ppm (Anonymous, 1997), which defines the reference value for the lettuce. The acceptable daily amount of the nitrate for an adult whose weight is 70 kg is approximately 60 ppm as defined by the scientific committee of the European Union which was formed with an objective of controlling food (Anonymous, 1999). Moreover, the acceptable daily nitrate amount of 0,85 mg/kg body weight defined by the EU is definitely tolerated.On the other hand, bio fertilizers increase nutrient uptake. Since bacteria produce organic acid, they decrease the pH; the mycorrhiza, on the other hand, facilitate nutrient intake since their hypha reach the areas where the roots cannot reach and increase the root surface area (Hanafy-Ahmed, 1996; Ruiz-Lozano, 1995; Zodape, 2001; Smith and Read, 1997; Vessey, 2003, Ikiz, 2002).

This study compares the use of bio-fertilizers against chemical fertilization in the head salad cultivation, and identifies its success in terms of yield, quality and mineral nutrients, especially for Bacteria (2) (*Arthrobacter* sp.) Algae (*Chlorella spp*) and Bacteria (1) (*Bacillus subtilis*) (bio-fertilizers that are environment-friendly and licensed for the organic cultivation). These increases in the head salad yield might be due to the fact that the bio fertilizer stimulates root growth (Carletti *et al.,* 1997) and enhances mineral uptake (Ruiz-Lozano *et al.,* 1995). Moreover, it may also be related with the yield increase in bio fertilizer-inoculated plants due to their ability to produce antibacterial and antifungal compounds, growth hormones and siderephores (Pandy and Kumar, 1990). Although the nitrate content accumulated on the leaves of the head salad cultivated with the bio-fertilizers are found to be higher than the chemical fertilizer application, these nitrate doses are fairly below the values set for the lettuce by the Turkish Food Codex Directive and the EU.

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