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Effects of amino acids and nitrogen fixing bacteria on quantitative yield and essential oil content of basil (*Ocimum basilicum*)

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Abstract

This experiment was done to evaluate the effects of amino acid spraying and nitrogen fixing bacteria inoculation on the yield and yield components of basil (Ocimum basilicum). The experiment was conducted during the growing season of 2012. The treatment groups consisted of nitrogen fixing bacteria (Control, Azotobacter, Azospirillum and Azotobacter+Azospirillum) and the sprays of amino acids (Control, Aminolforte, Aminolforte + Humiforte). The experimental design was a factorial experiment based on randomized complete block design (RCBD) with three replications. Nitrogen fixing bacteria and amino acid spraying successfully manipulates the growth of basil, resulting in beneficial changes in yield and yield components. Combined application of treatments can be helpful in improving of yield and essential oil in Ocimum basilicum.

1. Introduction

Sweet basil (Ocimum basilicum L.) is a well-known and appreciated spice and medicinal plant [22]. This plant is the most frequently grown species of Ocimum genus for seasoning and medicinal purposes. The basil herb is used as an aromatic spice, a component of curative herbal mixtures, as well as a source of essential oil and other biologically active substances [10, 16].

Sustainability of agricultural systems has become an important issue throughout the world. Many of the sustainability issues are related to the quality and time dependent changes of the soil [12]. It is well known that intensive cultivation has led to a rapid decline in organic matter and nutrient levels besides affecting the physical properties of soil. Conversely, the management practices with organic materials influence agricultural sustainability by improving physical, chemical and biological properties of soils [24].

Several types of studies in the last 20 years have shown a beneficial effect on crop plants by inoculation of seeds with Azospirillum strains [5, 28]. Inoculation of plants with Azospirillum and Azotobacter can result in a significant change in various plant growth parameters. Positive effects of inoculation have been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone [15, 25].

The asparagine and glutamine connect the two important metabolic cycles of the plant, the carbon and nitrogen cycles, and they have an influence both on sugars and on proteins. The glycine is an amino acid that inhibits the apparent photorespiration done by C3 [26]. The methionine is the ethylene precursor, it regulates the flowering and fruit ripening; the asparagine and the glutamine help in the nutrient transport and as reserve of nitrogen, besides being important in the pollination and fruit set. The tryptophan inhibits the precocious flower and fruit fall and it is important in the process of production of enzyme that catalyses synthesis reaction of auxin. The glutamic acid is important for the synthesis of the auxin and fruit set and the alanine for the germination and the pollen grains fertility [26]. The main objective of this study was to evaluate the effects of nitrogen fixing bacteria and amino acids spraying on yield and essential oil basil (Ocimum basilicum).

2. Methodology

The present study was conducted during the growing season of 2012 at the research fields of RAN Company in Firouzkouh, Iran. The geographical location of the experimental station was 35º 45´ N and 52º 44´ E with the altitude of 1930 m. The soil of the experimental region was loamy-clay with pH 7.6 (Table 1).

Table1. Chemical analysis of vermicompost used in the experiment.

Texture	pH	ECe ds/m	N %	K Ppm	P ppm	OC %	Fe mg/kg	Zn mg/kg	Mn mg/kg
Loamy-Clay	7.6	1.55	0.127	720	48	1.86	8	1.1	6.6

A 4×3 factorial experiment, arranged in a randomized complete blocks designed with three replications. The treatments consisted of 4 level of nitrogen fixing bacteria (control, Azotobacter, Azospirillum and Azotobacter + Azospirillum) and 3 level of amino acid spraying (control, Aminolforte, Aminolforte + Humiforte). Inoculation was carried out by dipping the seeds in the cells suspension of 108 CFU/ml for 15 min [8]. Nitrogen (20 kg/ha) was applied to the plots before planting as starter. Also, 50 kg/ha P2o5 and K20 were used according to the soil analysis. Amino acids spraying were done when plants reached to 4-6 leaves. The nitrogen content and kind of amino acids in the aminolforte and humiforte are shown in Table 2 and 3.

Table 2. Type and content of nitrogen in amino acids used in the study.

Supplementary	Aminolforte	Humiforte
Total nitrogen(N)	1.10 % w/w	6.00 % w/w
Uric nitrogen	0.80 % w/w	3.70 % w/w
Organic nitrogen	0.30 % w/w	0.30 % w/w
Organic matter	2.00 % w/w	0.30 % w/w
Ammoniac nitrogen	-	1.40 % w/w
Free amino acids	3750 mg/L	3750 mg/L

Table 3. General distribution of amino acids constitutes.

Aminogram	Distribution (%)
Glycine	1.80
Valine	5.10
Proline	8.40
Alanine	13.21
Aspartic Acid	4.50
Argenine	8.40
Glutamic Acid	0.90
Lysine	5.10
Lectine	16.51
Isolectine	4.50
Phenilalanine	5.10
Methionine	4.20
Serine	3.90
Thereonine	3.00
Histidine	3.00
Glycocoll	9.60
Tyrosine	1.50
Glutamine	0.90
Cystine	0.30
Other	0.80

Each experimental plot was 3 m long and 2.1 m wide with the spacing of 15 cm between the plants and 35 cm between the rows. There was a space of 50 cm between the plots and 3 meters between replications. Seeds were directly sown by hand into the field to a depth of 2 cm. There was no incidence of pest or disease on basil during the experiment. Weeding was done manually and the plots were irrigated weekly. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.
Data were recorded for the plant height, number of branches per plant, leaf yield, stem yield, total dry matter and essential oil content. Ten plants were randomly selected from each plot and the observations were recorded. At the flowering stage, the plant height, from plant base to the tip of plant, was measured for each plot using a ruler (±0.1 cm) [8]. Number of branches per plant was recorded at the end of growth season. In addition, For evaluating the total dry weight, plants were put in the oven at 75º C for 48 h and dry weight was calculated using a digital balance (Sartorius B310S; ±0.01 g) [3, 8]. In order to determine leaf and stem yield, leaves and stems for each plots were manually harvested following the air-drying of pods at 23-29° C [1].

2.1 Essential oil extraction

To determine the amount of essential oil, a sample of 200 g of aboveground parts of plants was mixed with 500 ml of tap water in a flask and the water was distilled for 3 h using a Clevenger-type apparatus. The oil content was measured based on ml oil per 100 g dry matter of aboveground parts at the Research Institute of forests and rangeland.

2.2 Statistical analysis

All the data were subjected to statistical analysis (one-way ANOVA) using SAS software [30]. Differences between the treatments were performed by Duncan's Multiple Range Test (DMRT) at 5% confidence interval. Transformations were applied to the data to assure that the residuals had normal distribution [29].

3. Results

3.1 Plant height

The present results have indicated that plant heights were not affected by the inoculation of seeds by nitrogen fixing bacteria. But, amino acid spraying could affect the basil height, significantly. Also, interaction of treatments caused significant differences on plant height (Table 4).
Mean comparison showed that the highest plant height (35.57 cm) obtained when aminolforte and humiforte sprayed on the plant, simultaneously (Table 5).

3.2 Number of branches per plant

The results have demonstrated that number of branches per plant was influenced by the inoculation of seeds by nitrogen fixing bacteria and amino acid spraying, significantly. Also, the results showed that the interaction of biofertilizers and amino acids was significant (Table 4). Among various treatments, the highest number of branches per plant (14.11 and 14.08) was obtained by using Azotobacter+Azospirillum and aminolforte+humiforte, respectively. Results of interaction effects of treatments showed that the highest number of branches per plant (15.38) was obtained at aminolforte+humiforte and inoculation seeds by Azotobacter.

Table 4. Analysis of variances for measured traits

Treatments	df	Height	Branches/Plant	Leaf Yield	Stem Yield	Total Dry Matter	Essential Oil
N	4	7.95	4.46*	601909.99**	881593.19**	50023.42**	0.01**
A	2	35.54**	5.44*	24270.47	569540.27**	7025.77	0.03**
N × A	6	12.86*	3.01*	729111.36**	61858.47*	36923.60**	0.04**
Error	24	62.66	1.14	68212.24	19831.24	6915.10	0.002
CV %	-	6.07	7.97	11.5	5.72	12.04	15.17

N: nitrogen fixing bacteria; A: amino acid

Table 5. Effects of nitrogen fixing bacteria and amino acid spraying on some traits of mungbean.

Treatments	Height	Branches/Plant	Leaf Yield	Stem Yield	Total Dry Matter	Essential Oil
Nitrogen Fixing Bacteria						
Control	32.91 a	13.22 a	2503.18 a	2415.54 bc	664.07 b	0.3 a
Azotobacter	34.37 a	12.45 b	2410.67 ab	2337.69 cd	705.11 a	0.33 a
A zospirillum	34.61 a	13.78 a	2119.78 b	2184.31 d	664.28 b	0.31 a
Azotobacter+Azospirillum	33.89 a	14.11 a	2533.38 a	2908.25 a	675.18 b	0.25 b
Amino acid						
Control	34.04 b	13.33 b	2545.81 a	2459.33 b	654.08 b	0.28 ab
Aminolforte	32.21 c	12.75 ab	2339.54 a	2244.65 c	622.13 b	0.35 a
Aminolforte+Humiforte	35.57 a	14.08 a	2312.57 a	2680.35 a	755.25 a	0.25 b

Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

3.3 Leaf yield

The results have demonstrated that leaf yield was influenced by bacteria inoculation. But, amino acid spraying could not affect leaf yield, significantly (Table 4). Among various treatments, the application of Azotobacter+Azospirillum has indicated maximum increase in leaf yield (2533.38 kg/ha). The present results showed that the interaction of treatments was significant (Table 4). The highest leaf yield was obtained at Azotobacter+Azospirillum and spraying aminolforte, simultaneously.

3.4 Stem yield

Results presented in Table 2 show that seed yield were influenced by bacteria inoculation and amino acid spraying (Table 4). Among various treatments, the application of Azotobacter+Azospirillum has indicated maximum increase in stem yield (2908.25 kg/ha). Also, spraying aminolforte+humiforte caused the highest stem yield (2680.35 kg/ha) (Table 5). The present results show that the interaction of treatments was significant (Table 4). The highest stem yield was obtained by Azotobacter inoculation and aminolforte+humiforte spraying.

3.5 Total dry matter

The present results show that thenitrogen fixing bacteria and interaction of nitrogen fixing bacteria and amino acid were significant (Table 4). The highest dry matter (821.89 kg/ha) was obtained when Azotobacter inoculated with seeds and aminolforte+humiforte sprayed.

3.6 Essential oil content

Analysis of variance showed that vermicompost and aminolforte had significant effects on the essential oil content (Table 4). Mean Comparison showed significant differences between various levels of nitrogen fixing bacteria treatment (Table 5). Total essential oil content varied between 0.25 and 0.33% (Table 3), which the highest value was obtained Azotobacter inoculation. There were significant differences in essential oil content between the plants sprayed with various levels of amino acids. Foliar application of aminolforte resulted in greatest essential oil content (Table 5).

4. Discussion

According to the present analysis, nitrogen fixing bacteria have shown positive effects on measured traits. Improved growth, development and height of crops have previously been reported by using Azotobacter and Azospirillum [8, 17, 25]. The present result was derived from the improvement of nitrogen fixing bacteria' activities in soil at Azotobacter+Azospirillum treatment.

Biofertilizer (nitrogen fixing bacteria), promoted yield through the enhancement of yield attributes. These results are in agreement with the investigation of Kumar et al. (2002) on Coriandrum sativum [17], Migahed et al. (2004) on Apium graveolens [19], Abdou et al. (2004) and Mahfouz and Sharaf Eldin (2007) on Foeniculum vulgare [1, 18] and Valadabadi and Farahani (2011) on Nigella sativa [27]. Results likely show that the positive and synergistic effect of interaction between two factors Many reports have shown that the interaction between biofertilizers can be beneficial for plant growth and yield [9, 20, 23, 27].

Amino acids are the fundamental ingredients of the process of protein synthesis because of their nitrogen content. The importance of nitrogen or amino acids came from their increased application for the biosynthesis of alarge variety of non-nitrogenous materials that is, pigments, vitamins, coenzymes, purine and pyrimidine bases [11].

Many studies have reported that foliar application of amino acids caused an increase in the growth and development of plants [2, 4, 11]. Neeraja et al. (2005) found that amino acid treatment increased the number of flowers, fruit setting and fruit yield in tomato [21].

The results clearly demonstrate the effectiveness of nitrogen fixing bacteria and amino acid spraying in increasing the quantitative yield. Nitrogen fixing bacteria increase the growth rate which leads to the biological yield improvement. This finding is in accordance with the previous observations [4, 7, 13].

Clearly, more experimental research aiming to specifically address nitrogen fixing bacteria application and amino acid spraying and their consequences for medicinal plants seems necessary.

5. Conclusion

It is clear from the present study that nitrogen fixing bacteria and amino acid spraying successfully manipulates the growth of basil, resulting in beneficial changes in yield and yield components. Combined application of nitrogen fixing bacteria and amino acid spraying can be helpful in developing of production and yield in Ocimum basilicum L.

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