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Investigation Effect of Different Level of Fertilizer and Biologic Fertilizer on Basil Varieties Crop Production at Greenhouse in Khuzestan Province (Southwest of Iran)

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Application of bio-fertilizers, especially the plant growth promoting bacteria, is most important strategy for the integrated management of the plant nutrition in the sustainable agriculture system with sufficient input.

OBJECTIVES: This study is aimed to examine the changes of plant production and Economic value of basil in response to apply different level of fertilizer and cultivar.

METHODS: This research was carried out via combined split plot in time experiment based on randomized complete blocks design with three replications along 2017-2018 year in greenhouse condition. Main factor included five fertilizer and bio fertilizer combinations [F_1: Humic acid (4 kg.ha^{-1}) + Complete fertilizer (N-P-K: 100-180-250), F_2: Phosphorus bio fertilizer (500 gr.ha^{-1}) + Complete fertilizer (N-P-K: 100-180-250), F_3: Humic acid (4 kg.ha^{-1}) + Phosphorus bio fertilizer (500 gr.ha^{-1}) + Complete fertilizer (N-P-K: 100-180-250), F_4: Humic acid (4 kg.ha^{-1}) + Phosphorus bio fertilizer (500 gr.ha^{-1}), F_5: Control (None use of Humic acid and Phosphorus bio fertilizer) contain Complete fertilizer (N-P-K: 100-180-250)]. The sub-factor was the basil variety, including green (C_1) and purple basil (C_2).

RESULT: According result of analysis of variance effect of different level of fertilizer and cultivar on studied traits was significant at 1% probability level. Interaction effect of treatments (instead fertilizer × cultivar) was not significant. Mean comparison of different level of fertilizer revealed F_4 had the highest amount of measured traits. Also C1 cultivar was superior to C_2 cultivar. Compare interaction effect of treatment showed C1 × Phosphorus bio fertilizer × Humic acid with nonuse complete fertilizer had the highest amount of studied traits.

CONCLUSION: Generally based on result of current research F_4 treatment [Humic acid (4 kg.ha^{-1}) + Phosphorus bio fertilizer (500 gr.ha^{-1})] by achieve 26.8 t.ha^{-1} wet yield, 2.1 t.ha^{-1} dry yield, 92.5 kg.m^{-3} and 48.5 Million Toman was the best treatment and can be advised to producers in studied region.

KEYWORDS: *Dry matter, Humic acid, Protein, Water use efficiency, Yield.*

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1. BACKGROUND

The most basic material need of humanity is the access to resources including air, food, and water in sufficient amounts. Although the use of chemical fertilizers adds large amounts of nutrients to the soil, plants are not able to absorb all these nutrients and materials so that the material accumulation over years has led to the current acute problems such as erosion, soil destruction, environmental pollutions, salt accumulation, and changes in pH of the soil and thus reduced fertility, creation of undesirable complexes, reduced levels of organic carbon, biodiversity loss, genetic erosion, and finally the disruption of the food chain (Dastmozd *et al*., 2015). Cheraghi *et al*. (2016) studied the effect of organic manure and phosphorus fertilizer on yield and yield components of bread wheat and reported that the combined application of organic manure or vermicompot with chemical fertilizer has a better effect on yield and yield components of common wheat rather than single application. On the other hand combined application of organic and chemical fertilizers had more efficiency due to some positive interaction between their microorganisms in the soil that led to a synergistic effect and therefore lead to an increase in seed yield. Shadab Niazi *et al*. (2017) by evaluate the effect of different level of vermicompost (0, 2.5 and 5 $t.ha^{-1}$) on mung bean, reported the highest protein yield and seed yield were obtained from 5 $t.ha^{-1}$ vermicompost and the least of these traits were due to non-use of vermicompost. Increase protein percentage with using bio-fertilizers is due to the effect of bacterial inoculation that increased the effective regulation of the growth, physiological and metabolic activity of the plant (Eidy Zadeh *et al*., 2012). Eydizadeh *et al*. (2010) stated that biological fertilizers increase the root contact with soil and ultimately increase the absorption of nutrients. Mentioned researchers also stated that the production of various acids by bacteria could lead to more organic solubility of the soil. It seems that the effect of bio-fertilizers provides up to 50% of the plant's nutritional requirements, and the rest of the plant's needs must be provided through the use of chemical fertilizers. Hojattipor *et al*. (2014) reported that the maximum total dry weight was obtained in wheat with increasing nitrogen fertilizer up to 225 $kg.ha^{-1}$, along with biological nitrogen fertilizer of nitrokara. Khuzestan province is located in a hot and semi-arid region. This province is suitable for the production of vegetables in terms of weather conditions. But due to lack of water, there are many limitations in the cultivation and production of vegetable and summer crops. Especially the method of irrigation in the fields is leaking by furrow irrigation method. One of the basic components of increasing the yield of agricultural products is the consumption of more inputs, especially chemical fertilizers. However, the increase in the use of chemical fertilizers in recent decades has led to serious environmental problems, which can be solved by replacing them with biological fertilizers.

2. OBJECTIVES

This research is aimed to examine the changes of plant production and Economic value of basil in response to apply different level of fertilizer and cultivar.

3. MATERIALS AND METHODS

3.1. Greenhouse and Treatments Information

In order to comprehensively assess the functional and morphological aspects of two basil (*Ocimum basilicum* L.) varieties under various nutritional regimes within a controlled greenhouse environment, an extensive field experiment was meticulously carried out in the northern region of Khuzestan Province, specifically within the Shushtar greenhouse complex. The main goal was to achieve a sustainable and productive basil crop while optimizing resource utilization. The principal factor under investigation in this research revolved around the application of biological fertilizers, which featured five distinct treatment groups, outlined as follows: Humic Acid Treatment: A profound emphasis was placed on the utilization of Humolife 95%, a high-quality humic acid product from SWISS IMO, with a substantial quantity of 4 kilograms per hectare. This humic acid application was complemented by a complete chemical fertilizer containing the key macro elements, namely nitrogen, phosphorus, and potassium, supplied at the ratio of 250-180-100 kilograms per hectare. For the sake of clarity, this treatment will be referred to as "Humic Acid." Phosphor2 Treatment: This treatment involved the application of 500 grams of Phosphor2, a noteworthy biological fertilizer from Zist Fanavar Sabz Company, per hectare. Similarly, this was accompanied by the concurrent use of a complete chemical fertilizer formulated with nitrogen, phosphorus, and potassium, applied at the ratio of 250-180-100 kilograms per hectare. This specific treatment is succinctly designated as "Phosphor2." Biological Fertilizers Treatment: An integral aspect of the study involved a multifaceted approach, combining the biological fertilizers Phosphor2 and Humic Acid with the previously mentioned complete chemical fertilizer. This holistic approach was geared towards assessing the combined influence of these biological fertilizers and the chemical fertilizer on basil growth. Biological Fertilizers Only: This treatment delved into the exclusive utilization of Phosphor2 and Humic Acid as the primary source of nutrition for the basil crops, with the omission of complete chemical fertilizers. Control Group: As the control in the study, this treatment strived to offer a baseline scenario by solely utilizing a complete chemical fertilizer at the standard ratio of 250-180-100 kilograms per hectare. Importantly, this treatment excluded the addition of Humic Acid and Phosphor2 biological fertilizers. Additionally, two diverse basil varieties, green and purple basil, were considered as a secondary factor in the research.

3.2. Greenhouse Management

The greenhouse employed for this study featured an aluminum frame structure with a towering height of 5 meters, thoughtfully designed with advanced environmental control elements such as fans and cooling pads to ensure optimal temperature and humidity. Furthermore, a high-pressure drip irrigation system utilizing T-tape tubing was meticulously implemented. Soil composition consisted of a light-textured substrate with the regular application of 40 tons of cattle and sheep manure per hectare annually. Additionally, 10% of the total volume was composed of sandy material, enhancing soil characteristics. The experimental procedure spanned over four growth cycles, each lasting 35 days and encompassing the duration from early September to late March, ensuring an in-depth evaluation of the basil crops.

3.3. Measured Traits

3.3.1. *Measurement of Raw Nitrogen and Protein Percentage*

To determine the raw nitrogen and crude protein percentages, the entire plant material was harvested, meticulously cleaned, and subjected to a drying process. The drying was executed within a controlled environment, with two different temperature regimens, namely around 50°C for one week or 70°C for 48 hours. After this drying process, the plant material was ground into a fine powder. The determination of the raw nitrogen content was achieved using an Atomic Absorption spectroscopy device, a technique based on the absorption of light by the atoms of the element being assessed. The raw nitrogen content was then quantified, and the approximate protein percentage was obtained by multiplying it by a factor of 6.25. The total protein content was computed by multiplying the raw protein percentage by the total dry matter content of the plant material (Bremner and Breitenbeck, 1983).

3.3.2. *Biological Efficiency and Water Use Efficiency (WUE)*

Biological efficiency and economic water use efficiency were calculated using established methodologies (Alizadeh, 2004; Hashemi Dezfuli, 1994) as indicated below:

Equ.1. Biological Efficiency ($kg.m^{-3}$): [Dry matter Yield ($kg.ha^{-1}$)]/[Total consumed Irrigation Water ($m^3.ha^{-1}$)].

Equ.2. Water Use Efficiency ($kg.m^{-3}$): [Harvested Marketable Product Yield per hectare ($kg.ha^{-1}$)] / [Total consumed Irrigation Water ($m^3.ha^{-1}$)].

Water Use Efficiency (WUE) is defined as the amount of produce achieved per unit volume of irrigation water used, typically measured in kilograms per cubic meter ($kg.m^{-3}$). The irrigation water volume was calculated based on the water output from the drip irrigation system during the irrigation period, with simultaneous measurements for accuracy. In order to calculate whether or not growing vegetables in the greenhouse is economical, considering the total fixed and variable costs during the period of using the greenhouse for each product and calculating the break-even point (The break-even point is the point where total costs and total revenue are equal and from that point onward, profitability can be achieved, in other words, any income that occurs after the break-even point from the sale of the product leads to profitability), the total profit from each treatment was calculated and statistically analyzed.

Equ. 3. Sales – VC- FC=0 or Q(P-v)-FC=0

Sales= Total sales

VC= Total variable costs of greenhouse and production facilities

FC= Total fixed costs of greenhouse and production facilities

Q= Total salable tonnage

P= Selling price per kilogram of vegetables

V= Variable cost per kilogram of vegetables sold

If we want to calculate the total salable tonnage at the break-even point, we use the following formula:

Equ. 4. Q = (FC + EBIT) / (p-v) = (FC + EBIT) / (CM$/unit)

Because the goal is to determine the break-even point, and at this point the net profit is zero, so instead of EBIT, zero is placed. After obtaining the break-even point for each treatment, the excess revenue from product sales is calculated and analyzed in statistical analysis (Mehrabi Bashrabadi, H. 2008).

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via MSTATC software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. *Yield*

According result of analysis of variance effect of fertilizer and cultivar on plant yield was significant at 1% probability level but interaction effect of treatments (instead fertilizer × cultivar at 1%) was not significant (Table 1). Mean comparison result of different level of fertilizer indicated that maximum plant yield (26.8 $t.ha^{-1}$) was noted for F_4 treatment and minimum of that (22.6 $t.ha^{-1}$) belonged to F_2 treatment (Table 2). The results of mean comparisons (Table 2) showed that among the cultivars, the highest yield (26.5 $t.ha^{-1}$) belonged to the green basil. Assessment mean comparison result of interaction effect of treatments showed maximum plant yield (29.3 $t.ha^{-1}$) was for Green Basil Phosphorus bio fertilizer and Humic acid with nonuse complete fertilizer and lowest one (21.3 $t.ha^{-1}$) belonged to Purple basil and Phosphorus bio fertilizer treatment (Table 2). Another researcher such as Khoshkhoi *et al.* (1991); Ranjbar (2013); Behbahani and Khayam Nekoui (2013); Bolandnazar *et al.* (2014); Gulshan (2017) reported same result.

4.2. *Total dry matter*

Result of analysis of variance revealed effect of fertilizer and cultivar on total dry matter was significant at 1% probability level but interaction effect of treatments (instead fertilizer × cultivar at 1%) was not significant (Table 1). According result of mean comparison maximum of total dry matter (2166 $kg.ha^{-1}$) was obtained for F_4 treatment and minimum of that (1942 $kg.ha^{-1}$) was for F_1 treatment (Table 2). The results of mean comparisons (Table 2) showed that among the cultivars, the highest total dry matter (2310 $kg.ha^{-1}$) belonged to the green basil.

Table 1. Result of combined analysis of variance of treatment on effective traits on yield

S.O.V	df	Yield ($t.ha^{-1}$)	Total dry matter ($kg.ha^{-1}$)	Protein yield ($kg.ha^{-1}$)	Water use efficiency ($kg.m^{-3}$)	Net profit (Million Toman)
Year	1	485385483.7^{ns}	648544.1^{ns}	70178.4^{ns}	13.6^{ns}	4249844352^{ns}
Replication	2	2243239.9	40598.9	4113.8	2.2	23433925
Fertilizer (F)	4	29797347.2**	107099.3**	13808.3**	1132.6**	260404639**
Y × F	4	55339777.2^{ns}	807052.3^{ns}	57570.9^{ns}	4.3^{ns}	452033591^{ns}
Cultivar (C)	1	260729260.4**	4109213.4**	640253.4**	1063.5**	2250006596**
Y × C	1	20644800.4^{ns}	1115206.7^{ns}	91104.1^{ns}	7.6^{ns}	178137524^{ns}
F × C	4	3785163.3**	69728.5**	27502.2**	126.7**	31517025**
Y × F ×C	4	12638433.3^{ns}	259984.3^{ns}	22847.4^{ns}	4.4^{ns}	100069663^{ns}
Error	36	6973627.5	33442.8	5424.9	35.8	65924358
CV (%)		10.81	8.93	12.25	8.16	19.95

^{ns}, * and ** : non-significant, significant at 1 and 5 % probability level, respectively.

Evaluation mean comparison result of interaction effect of treatments showed maximum total dry matter (2476 $kg.ha^{-1}$) was for Green Basil Phosphorus bio fertilizer and Humic acid with nonuse complete fertilizer and lowest one (1575 $kg.ha^{-1}$) belonged to Purple basil and Phosphorus bio fertilizer treatment (Table 2). Some researchers such as Chamani *et al*. (2012); Heidari and Khalili (2014); Madani *et al*. (2013) confirmed mentioned result.

4.3. *Protein yield*

According result of analysis of variance effect of fertilizer and cultivar on protein yield was significant at 1% probability level but interaction effect of treatments (instead fertilizer × cultivar at 1%) was not significant (Table 1). Mean comparison result of different level of fertilizer indicated that maximum protein yield (678 $kg.ha^{-1}$) was noted for F_4 treatment and minimum of that (561 $kg.ha^{-1}$) belonged to F_2 treatment (Table 2). The results of mean comparisons (Table 2) showed that among the cultivars, the highest protein yield (704 $kg.ha^{-1}$) belonged to the green basil. Assessment mean comparison result of interaction effect of treatments showed maximum protein yield (856 $kg.ha^{-1}$) was for Green Basil Phosphorus bio fertilizer and Humic acid with nonuse complete fertilizer and lowest one (456 $kg.ha^{-1}$) belonged to Purple basil and Phosphorus bio fertilizer treatment (Table 2). These findings were consistent with the results of the research of Kochaki *et al*. (2008), who stated that biofertilizers containing nitrogen-fixing and phosphate-dissolving bacteria had a useful and effective role in improving the growth characteristics, aerial organ function, and quality characteristics of this plant. Also, the results of this research are consistent with the results of Pour Hadi (2011) and Talai *et al*. (2014).

Table 2. Mean comparison effect of treatments on effective traits on yield

Treatment	Yield ($t.ha^{-1}$)	Total dry matter ($kg.ha^{-1}$)	Protein yield ($kg.ha^{-1}$)	Water use efficiency ($kg.m^{-3}$)	Net profit (Million Toman)
Fertilizer					
F_1	23.6b	1942b	605ab	81.5bc	36.3c
F_2	22.6c	1965b	561b	77.8c	30.9c
F_3	24.5ab	2061b	646a	84.6b	44.8b
F_4	26.8a	2166a	678a	92.5a	48.5a
F_5	24.7ab	2105a	572b	85b	48.4a
Cultivar					
C_1	26.5a	2310a	704a	91.5a	44.9a
C_2	22.4b	1786b	498b	77b	32.6b
Interaction effect of Fertilizer × Cultivar					
C1 × Phosphorus bio fertilizer	25.9bc	2309ab	756ab	89.4ab	42.8bc
C2 × Phosphorus bio fertilizer	21.3d	1575c	455c	73.7c	29.9e
C1 × Humic acid	23.7c	2150b	615b	82b	36.5cd
C2 × Humic acid	21.5d	1780bc	513bc	74.1c	29.9e
C1 × Humic acid × Phosphorus bio fertilizer	26.7b	2310ab	772ab	92.9ab	45.6b
C2 × Humic acid × Phosphorus bio fertilizer	22.4cd	1812b	521bc	77.1bc	32.6d
C1 × Phosphorus bio fertilizer × Humic acid with nonuse complete fertilizer	29.3a	2476a	856a	100.7a	52.9a
C2 × Phosphorus bio fertilizer × Humic acid with nonuse complete fertilizer	24.4bc	1858b	482c	84b	38.7c
C1 × Complete fertilizer	27.1ab	2305ab	625b	93.3ab	46.7b
C2 × Complete fertilizer	22.2cd	1907b	520bc	76.7bc	32.2d

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

F_1: Humic acid (4 $kg.ha^{-1}$) + complete fertilizer (N-P-K: 100-180-250).

F_2: Phosphorus bio fertilizer (500 $gr.ha^{-1}$) + complete fertilizer (N-P-K: 100-180-250).

F_3: Humic acid (4 $kg.ha^{-1}$) + Phosphorus bio fertilizer (500 $gr.ha^{-1}$) + complete fertilizer (N-P-K: 100-180-250).

F_4: Humic acid (4 $kg.ha^{-1}$) + Phosphorus bio fertilizer (500 $gr.ha^{-1}$).

F_5: Control (None use of Humic acid and Phosphorus bio fertilizer) contain complete fertilizer (N-P-K: 100-180-250).

C_1= Green Basil, **C_2**= Purple basil.

4.4. *Water Use Efficiency*

Result of analysis of variance revealed effect of fertilizer and cultivar on water use efficiency was significant at 1% probability level but interaction effect of treatments (instead fertilizer × cultivar at 1%) was not significant (Table 1). According result of mean comparison maximum of water use efficiency (92.5 $kg.m^{-3}$) was obtained for F_4 treatment and minimum of that (77.8 $kg. m^{-3}$) was for F_2 treatment (Table 2). The results of mean comparisons (Table 2) showed that among the cultivars, the highest water use efficiency (91.5 $kg.m^{-3}$) belonged to the green basil. Evaluation mean comparison result of interaction effect of treatments showed maximum water use efficiency (100.7 $kg.m^{-3}$) was for Green Basil Phosphorus bio fertilizer and Humic acid with nonuse complete fertilizer and lowest one (73.7

$kg.m^{-3}$) belonged to Purple basil and Phosphorus bio fertilizer treatment (Table 2). The results obtained from this experiment were consistent with the findings of other researchers such as Stroz *et al*. (2003); Gewaily *et al*. (2006) and Koochaki *et al*. (2008), they are reported the use of biological fertilizers is associated with an increase in water retention capacity, an increase in root volume, and as a result, an increase in plant growth speed.

4.5. *Net Profit of Basil Cultivation in Greenhouse*

Result of analysis of variance revealed effect of fertilizer and cultivar on net profit was significant at 1% probability level but interaction effect of treatments (instead fertilizer × cultivar at 1%) was not significant (Table 1). According result of mean comparison maximum of net profit (48.5 MT) was obtained for F_4 treatment and minimum of that (30.9 MT) was for F_2 treatment (Table 2). The results of mean comparisons (Table 2) showed that among the cultivars, the highest net profit (44.9 MT) belonged to the green basil. Evaluation mean comparison result of interaction effect of treatments showed maximum net profit (52.9 MT) was for Green Basil Phosphorus bio fertilizer and Humic acid with nonuse complete fertilizer and lowest one (29.9 MT) belonged to Purple basil and Phosphorus bio fertilizer treatment (Table 2). Due to the extreme fluctuations in the prices of products in the market, it is important to calculate the profit from growing vegetables, and in all the researches, obtaining profit is one of the main goals. The profitability results of the best treatments of this research were consistent with the results of Rezaei *et al*. (2015) and Fallahi *et al*. (2010).

5. CONCLUSION

Greenhouse cultivation provides an environment that allows for precise and controlled conditions, resulting in higher quantity and quality of produce compared to open-field cultivation. The more all contributing factors to production align in controlled conditions, the better the outcome. Successful outcomes are more likely when energy consumption adheres to established standards and when rigorous monitoring is applied at all stages of cultivation. In this experiment, the separate and combined application of biological fertilizers, such as fertile phosphorus and humic acid, increased the efficiency of greenhouse facilities. Humic acid, by increasing the production of nucleic acids and amino acids, enhances cellular proliferation throughout the plant, especially in the roots. Furthermore, humic acid modifies the physical properties and improves soil structure, providing increased water infiltration capacity. Biological fertilizers encompass various beneficial bacteria that are developed for specific purposes, such as nitrogen fixation and the release of phosphate ions, potassium, and iron from their insoluble compounds. These bacteria facilitate the uptake of essential plant nutrients, reduce plant diseases, improve soil structure, stimulate plant growth, and enhance both the quantity and quality of the yield. While this experiment was conducted in a traditional greenhouse with improved agricultural soil, the results were notably promising and informative. Water use efficiency in certain treatments and varieties was remarkably high, making it difficult to compare with open-field cultivation. Nonetheless, there is still room for improvement in soil conditions and nutrition to produce a healthier yield. Unfortunately, the rampant use of various chemical fertilizers and fungicides in greenhouse vegetable production often disregards proper nutritional principles and exceeds permissible limits. Activities in greenhouse cultivation should undoubtedly move forward in parallel with the objectives of producing healthy, organic products to achieve success in greenhouse operations, in addition to the goals of increasing yield.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

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