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Investigating the possibility of decrease consumption of chemical fertilizers with using the biofertilizers in Hyssopus officinalis in Iran

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ABSTRACT

The chemical fertilizers have harmful impact on the environment and decrease the quality of the agricultural products. This study was carried out in order to compare biological fertilizer type phosphate solubilizing bacterium (Barvar2) and nitrogen fixing bacterium (Nitroxen) and chemical fertilizer. The effects of biological and chemical fertilizers and mixtures of them were analyzed by using a complete random blocks design (CRD) with three replications during 2009 - 2011 in Arak. The effect of treatments were significant on certain characteristics such as plant height, number of branches, plant dry weight, biomass, essence percent, essence yield, LAI. Between the treatments $N_{150}P_{120}K_{150}$ with an average 9.314 kg ha^{-1} had highest essential oil yield and Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ with age 3469.51 kg ha^{-1} had highest biomass.

Key words: *Phosphate solubilizing bacteria, NPK, Nitrogen fixing bacteria, yield and essence.*

Introduction

Nitrogen, phosphor and potassium are the main elements needed for the plants. These elements were involved in all the biochemical process in the energizing compounds, as well as in the energy transferring mechanisms. Additionally, NPK are a part of the cell protein, playing a particular role as a part of the cell protein, cell membrane, nucleosides, which are responsible for reproduction and growth; despite its role in the herbal process and combination, the amount of phosphor in the herbal tissues is less than 0.1% of nitrogen. The damages resulting from consumption of chemical fertilizers are: reduction of the quantity and quality of crop – Boron aggregation, cadmium, and other heavy metals in the plant – reduction of absorption of copper, iron, and other micro elements by the root – destruction of the soil structure (1). Production and application of biological fertilizers as the most important practice in soil biotechnology was considered by capitalists of agriculture in all over the world. Biological fertilizers are consisting of useful bacteria and fungus that are produced for a special purpose, for example nitrogen fixing, releasing phosphate, potassium and iron ions from their insoluble compounds. These bacteria are usually around the root and help plant to uptake nutrients (2). It is obvious that these bacteria do not have just one role in soil. In addition helping nutrient uptake, helps plant to uptake other nutrients, decrease diseases, improve soil structure and consequently stimulate more plant growth and increase quantitative and qualitative yield (3). So, scientifically these bacteria are called "Plant growth promoting rhyzobacteria" or PGPR. Since these bacteria are exploited from the soil, they have a lot of benefits. Biological fertilizers are natural, so using these bacteria is considered as returning to the nature and exploiting natural components to improve it. Different studies indicated that biological fertilizers in addition of increasing nutrients uptake of plant, also they can improve plants growth and yield by plant hormones biosynthesis, controlling plant pathogens and some other mechanisms (4). Also it was understood in another experiment that these bacteria around the rhizosphere area can increase crop growth and yield by atmospheric nitrogen fixing, increasing nutrients access at rhizosphere area, increasing root contact area, and growth regulators production and improving useful symbiosis with host plant in different growth stages (5). Biological nitrogen application increased chamomile's flower yield. It was considerable in this experiment that capitulum yield (capitulum fresh and dry weight per hectare)

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by bio sulfur biological fertilizer was significantly more than other treatments (6). Growth promoting bacteria including Pseudomonas fluorescence, increased biomass yield and alkaloids content of plant periwinkle (Catharanthus roseus) (7). The presence of Pseudomonas, azotobacter and azospirillum was reported in environment of some medicinal plants such as periwinkle (Catharanthus roseus), Basil (Ocimum sanctum), Coleus Forskohlii and Aloe vera, so that, population of these bacteria in all of these medicinal plants species were more compared to other environments. Plant fresh weight was affected by biological fertilizers, so that, the most shoot fresh weight was acquired in super Nitro-plus treatment (8). A significant improve in grain yield of a medicinal plant from Euphorbaceae family with the scientific name of Phyllanthus amarus by applying phosphate solubilizing bacteria compared to control (9). Applying biological fertilizers containing Azotobacter and Azospirillum bacteria in medicinal plant sage (Salvia officinalis) increased plant height, shoot dry and fresh weight of this plant (10). On rosemary plant (Rosmarinus officinalis) azotobacter inoculation increased essential oil percentage of this plant (11). Integrating the application of mycorrhiza fungi with azospirillum and bacillus bacteria increased biomass production of Cymbopogon Martinii (12). Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13). Some studies have reported that plant growth promoter bacteria such as Azotobacter chroococcum, Azospirillum lipoferum, could cause increased growth and yield of medicinal plants such as rosemary (14), fennel (15), mint (16), geranium (17), marjoram (18), davana (19), dragonhead (20) black cumin (21), dill (22), thyme (23) and basil (24).

Hyssopus officinalis is a perennial shrub plant. This plant possesses a direct root with plenty of branches; the stem of this plan is square and direct, its height is 50 to 70cm. The length of the leaf of Hyssop is 2 to 4cm, and its width is 0.5 to 1cm, which rests along the stem (as the shape of cross). The flowers of Hyssop are white – pink – blue and mixed colors; the amount of essence on the vegetative structure of Hyssop varies between 0.3 and 1%, and the maximum amount is in the head of flower branch. The key compounds of the essence are 50% of pinocamphone; other key compounds are alfa and beta pinene, camphone, myrcene – terpineol and thujene (25).

Material and methods

This study was carried out during 2009-2011 on a land with area of 2000m in Arak on a field with geographical coordinates: latitude: 34º- 5', longitude: 49º- 42', and height of 1787 m above the sea level. The suitable land was chosen and after preparation of the land the seeds were planted in the plot. The depth of seeds planted on the furrow was 1 to 2cm. The intervals between plants are considered 40cm. Generally, the number of plants on each plot is considered 50. Based on the soil test the amount of nitrogen and potassium fertilizers needed were 100 kg per hectare and phosphor fertilizer 80 kg per hectare. Of course, the seeds before being planted, they were saturated with Component of material design Phosphate solubilizing bacteria (Barvar2) and Nitrogen fixing bacteria (Nitroxen) as per the prescribed method. Sampling for measuring the physiological procedure was commenced 25 days upon greening and was repeated once every week.

Table 1- Field Soil Test

Depth	% of saturation	Ec	Ph	T.N.V	O.M (%)	P	K	N	Sand	silt	clay	Mn	Cu	Zn	Fe
0-30	37	0.6	8	28	0.61	11.4	434	6.2	52	36	12	10.49	2.16	2.96	7.88

Sampling was carried out in such way: two side lines were removed from each plot due to marginal impacts, and after removing one bush from the three middle lines (from both ends of the cultivation line), 6 bushes was picked from each plot for phonology and 10 bushes were sent to the

lab to measure the percentage of the essence (quantitative).

After collecting samples, their roots, stems, leaves and twigs were separated and each weighed individually. Then for 72 hours were placed in oven at 75°C. After being dried, the samples were weighed again. After harvesting, a number of samples were dried in shadow and extraction was performed in the laboratory. To measure the essential oil amount, Clevenger apparatus and water distillation method was used; 100 grams of leaf powder and dried shoot was poured in 1-liter flask of device and 800 ml water added to it and heated for 2 hours.

Statistical analyses of the data based on complete randomize block and comparing their average by Duncan test, analyzing the data using SAS 9.1 software.

Results and Discussion:

Plant height: The table analyze of variance showed that the year and treatments had a significant effects on plant height at 0.01 levels. Among the treatments $N_{150}P_{120}K_{150}$ (150% recommended) with an average 53.42 cm had highest and control with 38.81 cm had least amount. Results show that the application mixed of the biofertilizer alone or both with 50% chemical fertilizer have leaded into significant increase in plant height comparing with control and did not show a significant difference with $N_{150}P_{120}K_{150}$ treatment. Applying biological fertilizers containing Azotobacter and Azospirillum bacteria in medicinal plant sage (Salvia officinalis) increased plant height of this plant (10). Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13).

Number of branches: Investigating analyze of variance table show that treatments had a significant effects on number of branches at 0.01 levels. Among the treatments Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ with an average 20.56 braches had highest and control with 11.22 beaches had least amount. Results show that the application mixed of biofertilizer alone or both with 50% chemical fertilizer has leaded into significant increase number of branches per plant compare to control and did not show a significant difference with $N_{150}P_{120}K_{150}$ treatment. Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13).

Plant dry weight: The Study for analyze of variance table showed that year and treatments both had a significant effects on plant dry weight at 0.01 levels. Among the treatments Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ with an average 55.51 g had highest and control with 33.40 g had least amount. Results show that the mixed application of biofertilizer alone or both with 50% chemical fertilizer has leaded into significant increase in plant dry weight compare to control and had a significant increase with chemical fertilizers treatments. Plant fresh weight was affected by biological fertilizers, so that, the most shoot fresh weight was acquired in super Nitro-plus treatment (8). Applying biological fertilizers containing Azotobacter and Azospirillum bacteria in medicinal plant sage (Salvia officinalis) increased shoot dry and fresh weight of this plant (10). Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13).

Canopy: The Analyze variance table showed that year and treatments had a significant affects on canopy at 0.01and 0.05 levels. Among the treatments Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ with an average 618.96 cm^2 had highest and control with 369.41 cm^2 had least amount. Results show that the mixed application of biofertilizer alone or both with 50% chemical fertilizer has leaded in to a significant increase in canopy compare to control and did not show a significant difference with chemical fertilizers treatments. Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13).

Essential oil percent: The Study of analyze variance table showed that treatments had a significant effects on Essential oil percent at 0.01 levels. Among the treatments $N_{150}P_{120}K_{150}$ with an average of 2.87% had highest and control with 1.51% had least amount. Results show that application mixed of biofertilizer alone or both with 50% chemical fertilizer had leaded in to a significant increase in essential oil percent compare to control but they showed a significant decrease with $N_{150}P_{120}K_{150}$ treatment. Growth promoting bacteria including Pseudomonas fluorescence, increased alkaloids content of plant periwinkle (Catharanthus roseus) (7). A significant improve in grain yield of a medicinal plant from Euphorbaceae

family with the scientific name of Phyllanthus amarus by applying phosphate solubilizing bacteria compared to control (9). On rosemary plant (Rosmarinus officinalis) azotobacter inoculation increased essential oil percentage of this plant (11).

Biomass: Analyze variance table showed that year and treatments had a significant effects on Biomass at 0.01levels. Among the treatments Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ with an average 3469.51 kg ha^{-1} had highest and control with 2087.61 kg ha^{-1} had least amount. Results show that application mixed of biofertilizer alone or both with 50% chemical fertilizer has leaded in to a significant increase in Biomass compare to control and Barvar2+Nitroxen+ $N_{50}P_{40}K_{50}$ showed a significantly increased compared to chemical fertilizers treatments.

Biological nitrogen application increased chamomile's flower yield. It was considerable in this experiment that capitulum yield (capitulum fresh and dry weight per hectare) by bio sulfur biological fertilizer was significantly more than other treatments (6). Growth promoting bacteria including Pseudomonas fluorescence, increased biomass yield of plant periwinkle (Catharanthus roseus) (7). Plant fresh weight was affected by biological fertilizers, so that, the most shoot fresh weight was acquired in super Nitro-plus treatment (8). A significant improve in grain yield of a medicinal plant from Euphorbaceae family with the scientific name of Phyllanthus amarus by applying phosphate solubilizing bacteria compared to control (9). Applying biological fertilizers containing Azotobacter and Azospirillum bacteria in medicinal plant sage (Salvia officinalis) increased shoot dry and fresh weight of this plant (10). Integrating the application of mycorrhiza fungi with azospirillum and bacillus bacteria increased biomass production of Cymbopogon Martinii (12). Bio-fertilizers application increased plant growth significantly in Thymus vulgaris (13).

Essential oil yield: The Study analyze of variance table showed that treatments had a significant effects on essential oil yield at 0.01 levels. Between the treatments $N_{150}P_{120}K_{150}$ with an average of 9.314 kg ha^{-1} had highest and control with 3.718 kg ha^{-1} had least amount. Results show that application mixed of biofertilizer alone or both with 50% chemical fertilizer has leaded in to a significant increase in essential oil yield compare to control. Some studies have reported that plant growth promoter bacteria such as Azotobacter chroococcum, Azospirillum lipoferum, could cause increased growth and yield of medicinal plants such as rosemary (14), fennel (15), mint (16), geranium (17), marjoram (18), davana (19), dragonhead (20) black cumin (21), dill (22), thyme (23) and basil (24).

Leaf Area Index: The Study analyze of variance table showed that treatments had a significant effects on LAI at 0.01 levels. Among the treatments control with an average of 6.33 had highest and Barvar2 with 4.81 had least amount.

Table 2: Analysis of variance for hyssop influence of biological and chemical fertilizers

* Significant difference at α = 0.05 ** significant difference at α = 0.01 ns: no significant difference.

Sources of variations (S.O.V)	df	Mean Square of mean characteristics							
		Plant height	Number of branches	Plant dry weight	Canopy	Essence percent	Biomass	Essence yield	Leaf area index
Year (Y)	2	962.845**	27.5440 ns	2037.38 **	135576 *	0.232 ns	7958541.3 **	9.6700 ns	16.538 *
Error a	6	75.943	24.522	160.772	38942.4	1.444	628015.8	37.953	4.028
Treatment (T)	9	295.534 **	112.298 **	414.691 **	58048.7 **	1.716 **	1619886.1 **	27.451 **	1.8440 ns
Interaction Y×T	18	34.8970 ns	17.0750 *	60.9760 ns	10069.0 ns	0.117 ns	238186.40 ns	2.7450 ns	1.9450 ns
Error b	54	23.003	7.49800	47.4310	15944.7	0.122	185277.14	2.6460	1.6170

Table 3: Comparing the Mean characteristics of Hyssop biological and chemical fertilizers influence

Treatments	Average of characteristics							
	Plant height	Number branches	Plant dry weight	Canopy (cm^2)	Essence (%)	Biomass ($kgha^{-1}$)	Essence ($kgha^{-1}$)	LAI
Control	38.81 c	11.22 e	33.40 c	369.41 d	1.51 f	2087.6 c	3.718 e	6.33 a
Barvar2	40.47 c	11.89 e	33.57 c	423.02 cd	1.55 ef	2098.4 c	3.911 e	4.81 b
Nitrogen	41.63 c	12.00 e	33.77 c	440.45 cd	1.58 ef	2111.2 c	4.578 de	4.91 b
Barvar2+ Nitrogen	42.08 c	13.56 de	40.47 bc	476.39 bcd	1.75 def	2529.8 bc	4.597 de	4.93 b
$N_{50}P_{40}K_{50}$	46.62 b	14.78 cd	38.12 bc	473.55 bcd	1.66 def	2382.7 bc	5.137 cde	5.18 ab
$N_{100}P_{80}K_{100}$	48.51 ab	18.00 ab	45.18 b	540.05 abc	2.27 bc	2823.8 b	6.387 bc	5.45 ab
$N_{150}P_{120}K_{150}$	53.42 a	18.11 ab	40.16 bc	521.04 abc	2.87 a	2510.1 bc	9.314 a	5.62 ab
Barvar2+ $N_{50}P_{40}K_{50}$	51.63 a	16.89 bc	41.42 b	606.01 ab	1.95 cd	2588.8 b	6.049 bcd	5.49 ab
Nitrogen+$N_{50}P_{40}K_{50}$	53.02 a	20.44 a	44.67 b	553.66 abc	1.90 de	2792.3 c	6.892 b	5.56 ab
Barvar2+ Nitrogen+ $N_{50}P_{40}K_{50}$	52.61 a	20.56 a	55.51 a	618.96 a	2.34 b	3469.5 a	7.291 b	5.59 ab

There is no significant difference among the mean with the same letters

Conclusion

Mixture application of bio fertilizers bacteria and 50% of recommended chemical fertilizer could have a significant effect on characteristic of plant height, number of stems, plant dry weight, canopy, essential oil percent, biomass and essential oil yield and cause a Significant increase in characteristics compared to control. Also in most of characteristics different levels of chemical fertilizer were higher or equal to them.

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