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Original Article

Effect of Chemical and Biological Phosphorus on the Yield of Summer Savory (*Satureja hortensis* L.)

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Abstract

To survey the effects of chemical and biological phosphorus on the yield and essential oil of *Satureja hortensis* L. as an important medicinal plant, factorial experiment was conducted based on randomized complete block design with three replicates at Research Station of Faculty of Agriculture, Urmia University in 2011. The treatments were chemical phosphorus fertilizer with four levels (0, 50, 100 and 150 kg/ha P as triple supper phosphate) and biological phosphorus fertilizer with four levels (0, 100, 200 and 300 g/ha *Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5). The results of ANOVA showed the significant interaction effect between chemical and biological phosphorus on the yield of fresh weight, biological yield, stem dry weight, and the yield of drug and essential oil. There was non-significant effect of chemical and biological phosphorus on the essential oil percent, harvest index of drug and harvest index of essential oil. The highest amounts of total fresh weight (23327 kg/ha), biological yield (5282 kg/ha), stem dry weight (2523 kg/ha) and yield of drug (2759 kg/ha) and essential oil (79 kg/ha) were obtained from 200 g/ha of biological phosphorus without chemical fertilizer. The minimum amounts of total fresh weight, biological yield, stem dry weight, the yield of drug and essential oil were observed in higher than 200 g/ha of biological phosphorus.

Keywords: Biological phosphorus, Chemical phosphorus, Essential oil, Harvest index, *Satureja hortensis*, Yield

Introduction

The genus *Satureja* L. (Labiatae) comprises over 30 species with wide distribution in the Mediterranean region [1]. Fourteen species of *Satureja* are growing wild in Western and Northern parts of Iran [2]. *Satureja hortensis* (Summer Savory or Garden Savory) is a well-known annual and herbaceous aromatic plant. The stem is erect, richly branching from the base and 30-60 cm high. The flowers are bisexual with purplish-pink color [3]. It has been traditionally used as a stimulant, stomachic, carminative, expectorant, antidiarrheic, and aphrodisiac. Nowadays, *S. hortensis* is cultivated in large scale in Germany, French, Hungary and Spain [3] because of some Pharmaceutical properties such as anti-diarrheal and antispasmodic [4], anti-inflammatory, as well as antimicrobial properties [5,6].

The role of phosphorus (P) for plant growth has been intensively investigated [7-9] as an important plant nutrient, phosphorus regulates many physiological processes which affect yield components. They also play an active role in biologic control of plant pathogens [10]. Within conventional agriculture the mentioned issue has been solved with application of chemical fertilizers [11].

But in cultivation of medicinal plants, the real value is given to the quality while yield quantity comes in second step of importance. Some in concern of medicinal plants in natural and agro-ecosystems

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showed that sustainable agricultural approaches are the best methods in which these plants revealed better performance on the account of the harmony with nature. Therefore, global approach to medicinal plant production is leading toward sustainable agricultural systems. In addition, environmental impacts which are caused by over application of chemical fertilizers, energies and expenses of their production are the reasons for global tendering toward application of bio-fertilizers [12]. Phosphate solubilizing microorganisms are able to solubilize organic and inorganic phosphorus compounds by producing organic acid or phosphatase enzyme [13]. *Bacillus polymixa* and some species of *Pseudomonas* dissolve rock phosphates and convert them to solubilized forms. They stimulate plant growth synthesis of hormones and increase phosphorous intake and nitrogen fixation [14-16]. Thus, the main objective of this study was to investigate the effects of different amounts of phosphate solvent bacteria and chemical phosphorus fertilizers on the growth and yield of *Satureja hortensis* in Urmia condition.

Materials and Methods

The experiment was conducted in research field of Urmia University with latitude of 37.53 °N, 45.08 °E and 1320 m above sea level in 2011. The soil texture of experimental site was clay-loam (28% silt, 33% clay, 40% sand) with 22.5% felid capacity, 1.54 g cm^{-3} soil density, 1.98% organic matter, pH=7.6. The experiment was carried out in factorial based on randomized complete block design with three replications. The factors were chemical phosphorus with four levels (0, 50, 100 and 150 kg/ha P originated from triple supper phosphate) and biological phosphorus with four levels (0, 100, 200 and 300 g/ha of *Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5 in same ratio).

Summer savory (*Satureja hortensis*) seeds were inoculated with mixed of two species of phosphorus solvent bacteria (*Pseudomonas putida* Strain P13 and *Bacillus lentus* Strain P5) and they were sown. The experimental units in each block comprised of 6 lines of 3 meters long. Row to row and plant to plant spacing was 0.30 and 0.10 m, respectively. Chemical phosphorus (0, 50, 100 and 150 kg/ha of triple supper phosphate) was incorporated into the soil by hand, at the sowing time.

To obtain the yield of fresh weight, all of aerial parts of savory plants were completely harvested and were immediately weigh. To measure biological yield and the yield of drug and stem, different parts of aerial were separated to stem and drug, and then dried at 25 °C. Harvest index was calculated as drug and essential oil divided by total above-ground plant dry weight (biological yield). The air-dried parts of drug (25 g of the dry sample) were hydro-distilled in a Clevenger-type apparatus in 1000 ml round bottomed flask with 600 mL de ionized water for 3 hours.

Statistical analysis

Statistical evaluation was performed using MSTATC software. The effects of phosphorous chemical fertilizer and biological phosphorus as well as the interactions of these two factors were analyzed with the analysis of variance. The results of statistical analysis are expressed by F-values; asterisks indicate p-values: $P \leq 0.05$ and $P \leq 0.01$. The comparison of means carried out with Duncan's Multiple Range Test.

Results and Discussion

The results showed the significant interaction effect between chemical phosphorous and biological phosphorus on fresh weight (kg/ha), biological yield (kg/ha), stem dry weight (kg/ha), drug yield (kg/ha) and essential oil yield (kg/ha) ($P \leq 0.01$). There was no significant effect of chemical phosphorous and biological phosphorus on the percent of essential oil, harvest index of drug and essential oil (Table 1).

Means comparison indicated that the highest yield of fresh weight (23330 kg/ha) was obtained from 200 g/ha of biological phosphorous without chemical phosphorus. The lowest yield of fresh weight (9148 kg/ha) belonged to 50 kg//ha of chemical fertilizer and 300 g/ha biological phosphorus. Biological phosphorus caused to increase the yield of fresh weight by 200 and 100 g/ha to maximum value in 0 and 50 kg/ha of chemical phosphorus, respectively. But in higher levels of chemical phosphorus (100 kg/ha), not only the yield of fresh weight was not increased, but also it was decreased by 300 g/ha of biological phosphorus. In 100 and 150 kg/ha chemical fertilizer (higher amounts), 200 g/ha of biofertilizer level produced the higher yield than control treatment (without fertilizer).

Table 1 Analysis of variance the yield and essential oil of *Satureja hortensis* L. as affected by chemical phosphorous and biological phosphorus

Source of variation	df	Mean square (MS) Yield: Fresh weight	Biological	Stem dry weight	Drug	Essential oil	Essential oil percent	Drug Harvest index	Essential oil Harvest index
Replication	2	14950137	1303167	696281.0	194609.6	237.0	0.084	0.0095	13.905
Chemical phosphorus (A)	3	57128113**	3418224**	6880027.9**	975169.9**	788.5**	0.016^{ns}	0.00033^{ns}	1.004^{ns}
Biological phosphorus (B)	3	82053797**	5218433**	1026148.7**	16105003.2**	1232.7**	0.055^{ns}	0.001^{ns}	1.314^{ns}
A×B	9	31630941**	1590095**	416400.6**	393524.1**	318.1**	0.083^{ns}	0.00088^{ns}	3.191^{ns}
Error	30	4105984	252654	93717.8	55330.7	75.8	0.064	0.001	2.334
Coefficient of variation (%)		13.22	13.75	18.15	12.06	15.99	9.07	5.91	10.19

^{ns}, *, and **, non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. df. Degree of freedom.

In all amounts of chemical fertilizers, application of 300 g/ha biofertilizer led to significant reduction of yield. Application of 100 kg/ha chemical phosphorus raised the yield of fresh weight to more than control but it was less than the yield produced by 200 g/ha biofertilizer (Fig. 1).

Changes in biological yield like to fresh yield, was affected by fertile phosphorus, so the highest biological yield (5282 kg/ha) was obtained from 200 g/ha biological phosphorus without chemical fertilizer. And the lowest yield (2050 kg/ha) was observed at 300 g/ha biological fertilizer with 50 kg/ha chemical phosphorus. 200 and 100 g/ha of biological fertilizer on 0 and 50 kg/ha chemical phosphorus caused to significant rise of biological yield, respectively. Application of 100 kg/ha chemical phosphorus had the same yield by 0 and 200 g/ha biological fertilizer, followed down by 300 g/ha. Application of 150 kg/ha chemical phosphorus supply the much more amount of phosphorus, so biological fertilizer (phosphorus solvent bacteria) had no significant effect on biological yield (Fig. 2).

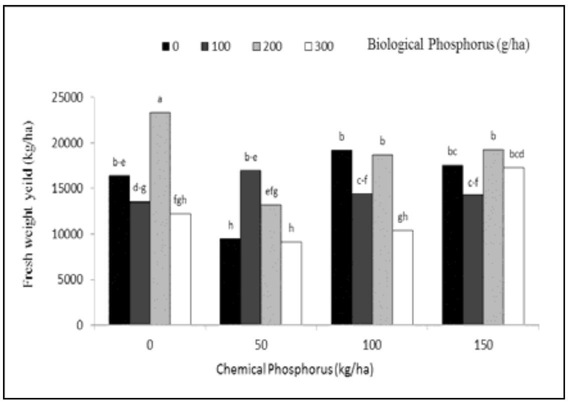

Fig. 1 Effect of chemical phosphorus and biological phosphorus on total fresh weight of *Satureja hortensis* L. and its significant differences Using Duncan Multiple Range Test.

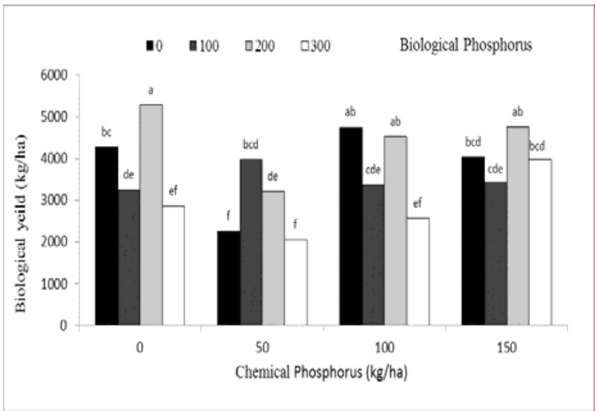

Fig. 2 Effect of chemical phosphorus and biological phosphorus on biological yield of *Satureja hortensis* L. and its significant differences Using Duncan Multiple Range Test.

The changes in stem dry weight were the same as biological yield. So, 200 g/ha of biological phosphorus fertilizer and control treatment of chemical phosphorus produced the highest yield of stem (2523 kg/ha) as well as 100 g/ha biological phosphorus in 50 kg/ha chemical treatment. The higher amounts of phosphorus, 100 and 150 kg/ha, were excess for *Satureja hortensis*. These amounts were enough to produce the yield of stem as well as higher than control treatments (Fig. 3).

Means comparison of drug yield indicated that the highest yield (2759 kg/ha) was obtained from 200 g/ha biological phosphorous without chemical fertilizer. The minimum yield of drug (1081 kg/ha) belonged to 50 kg//ha chemical phosphorus and 300 g/ha biological fertilizer. Biological phosphorus caused to enhance the yield of drug in little amounts of chemical phosphorus. But the massive uses of chemical phosphorus led to reduce the benefits of biological phosphorus (Phosphate solvent bacteria) (Fig. 4).

The highest yield of essential oil (79 kg/ha) was obtained from 200 g/ha of biological phosphorous and 150 kg/ha chemical fertilizer. The minimum yield of essential oil (30 kg/ha) belonged to 50 kg/ha of chemical and 300 g/ha biological phosphorus (Fig. 5).

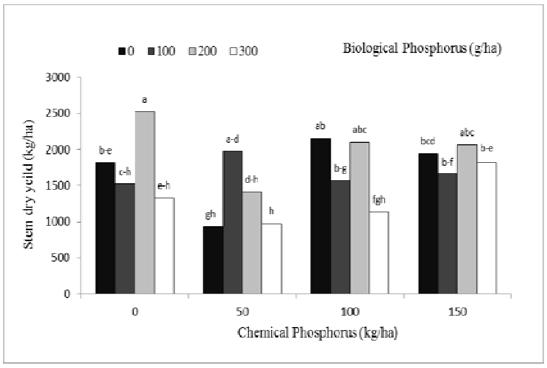

Fig. 3 Effect of chemical phosphorus and biological phosphorus on stem yield of *Satureja hortensis* L. and its significant differences Using Duncan Multiple Range Test.

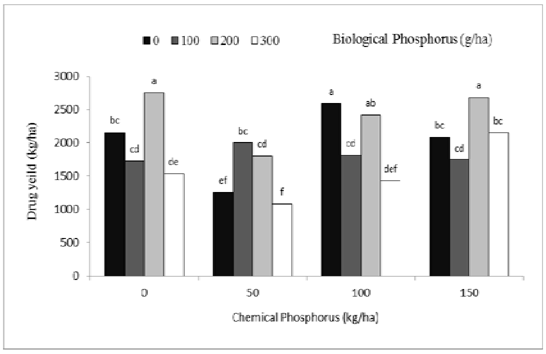

Fig. 4 Effect of chemical phosphorus and biological phosphorus on drug yield of *Satureja hortensis* L. and its significant differences Using Duncan Multiple Range Test.

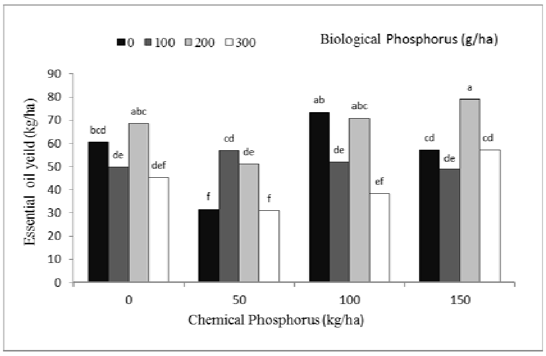

Fig. 5 Effect of chemical phosphorus and biological phosphorus on essential oil yield of *Satureja hortensis*L. and its significant differences Using Duncan Multiple Range Test.

In higher applications of chemical phosphorus (100 and 150 kg/ha), because of enough supply of nutrient (phosphorus), use of biological phosphorus did not affect the yield of essential oil. But in 50 kg/ha of chemical phosphorus, 100 and 200 g/ha of bio-fertilizer caused to increase the yield of essential oil followed down by additional bio-fertilizer. Large amount of phosphorus applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils [17, 18] like this study situation. Higher biomass, drug and essential oil production in biological and chemical phosphorus providing (Fig.s 1-5) were observed due to release of P by phosphate solubilizing bacteria (PSB) from insoluble / fixed forms and chemical feeding of phosphorus. There are strong evidences that soil bacteria are capable of transforming soil P to the forms available to plant [19]. In *Hyssopus officinalis,* plant performance was better with application of Super Nitro Plus (containing *Azospirillum / Bacillus subtilis / Pseudomonas fluorescens*), and mixtures of *Glomus intraradices* and *Pseudomonas fluorescens* in terms of most plant growth indices [20], because of providing nutrients.

Inoculation of Barley (*Hordeum vulgare* L.) seeds with sole bacteria positively affected the number of the number of seeds per plant, 1000-seed weight, biological yield and leaf chlorophyll content. However, application of bacteria in absence of any chemical phosphorus fertilizer had an appropriate performance and could increase biomass production to an acceptable level [21], so it (200 g/ha of biofertilizer) could be considered as a suitable substitute for chemical phosphorous fertilizer (100 and 150 kg/ha chemical phosphorus) in organic agricultural systems (Fig. 4 and 5).

According to results of Yazdani *et al.* [22] all treatments of phosphate solubilizing bacteria could reduce P application by 50% without any significant reduction of grain yield in maize.

Application of phosphate solubilizing bacteria and Arbuscular Mycorrhizal Fungi (AMF) due to increasing phosphorus uptake increased shoot dry matter yield, grain number in spike and grain yield of wheat by 52, 19 and 26% compared to the control, respectively.

Phosphorus application increased Olsen-P, Ca_2-P and Ca_8-P% while biological fertilizers reduced the amount of Ca_2-P and Ca_8-P% as unavailable phosphorus [23].

Phosphorus solubilizing bacteria increased average total dry matter of rapeseed from 7.5 (obtained from control) to 11.4 t/ha, seed oil content from 48.4 to 49.5% and grain yield from 3.0 to 9.9 t/ha. This raising up can be occurred because of enhance P concentration in the shoot dry matter from 0.21 to 0.78% by application of 125 kg mineral P /ha and phosphorus solubilizing bacteria [24]. According to Darzi *et al.* [25], Phosphate solubilizing bacterium increased significantly seed and biological yield in anise (*Pimpinella anisum* L.).

Establishment of an efficient inoculation of *Matricaria chamomilla* as an important medicinal plant, with phosphate solubilizing bacteria and application of triple super phosphate in proper amount (50 kg/ha) resulted in augmentation in essential oil yield and its components like chamauzulen and bisabolen [9]. The highest values of plant height, numbers of sub stem, stem size (length and diameter), leaf area and leaf dry weight of *Satureja hortensis* had been reported in higher amounts of biological or chemical phosphorus. At all, biological phosphorus (Phosphate solubilizing bacteria) can compensate the lack of chemical phosphorus fertilizer for plant growth (Fig. 1-5), that reported in previous researches [9, 24, 26].

Conclusion

Significant interaction effect of chemical×biological phosphorus indicated the varying response of summer savory plant to P solvent bacteria in different amounts of chemical phosphorus. And it could be efficiently used to improve fresh weight yield, biological yield, stem dry weight, drug yield and essential oil yield. According to our results, applications of biological phosphorus by 200 g/ha for all amounts of chemical phosphorus improve the yield of *Satureja hortensis*. While 100 kg/ha chemical phosphorus was enough to produce the highest yield as same as 200 g/ha of biological phosphorus singly and simultaneous.

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