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Original Article

Effects of Chemical and Biofertilizers on Yield and Production Factors of Valerian (*Valeriana officinalis* L.)

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ABSTRACT

A field experiment was conducted to evaluate the effects of bio and chemical fertilizers on yield and production factors of valerian (*Valeriana officinalis* L.) at the Homad-Absard Agricultural Complex in Damavand city, Tehran Province, Iran, through a factorial experiment based on a randomized complete blocks design with 3 replications during 2017 to 2020. Experimental factors consist Nitroxin and Phosphate Barvar2 biofertilizers each with 2 levels of inoculation and control, and also the urea (46% N) chemical fertilizer in 5 levels (0, 30, 60, 90 and 150 kg/ha). Measured parameters were root diameter (mm), root length (cm), leaf width (cm) and leaf length (cm) root dry weight (kg/ha), shoot dry weight (kg/ha), valernic acid content (%) and valernic acid yield (g/ha). Results showed that the biofertilizers had significant effect ($p \leq 0.01$) on the all quantity and quality parameters studied. Unlike the urea chemical fertilizer at all levels which had a negative effect on the yield and amount of valernic acid (%), biofertilizers increased it. Maximum and minimum amount of valernic acid (%) were measured in control (0.465%) and 150 kg/ha urea (0.215%) respectively. The highest and lowest root dry weight were obtained in interaction of Nitroxin+Phosphate barvar2 + 150 kg urea (665 kg/ha) and control (221 kg/ha) respectively. Results of this experiment showed that biofertilizers with positive effects on experimental parameters are able to replace the chemical fertilizers.

INTRODUCTION

Recently, WHO (World Health Organization) estimated that medicinal and aromatic plants are used by 80 percent of global population for some aspect of their primary health care needs [1]. World Health Organization (WHO) recognized the potential of traditional and alternate systems of medicine to provide succor to the health security of developing nations. According to WHO, around 28,000 wild or cultivated plant species have the potential for being used as medicinal plants, although fewer than 16% (4,478) of them used as a medicinal regulatory publication [1-3], with only 900 under cultivation and harvested from the wild. Global imports and exports (2000-2008) of medicinal plants were worth US$ 1.59 and 1.14 billion/year, respectively with >40%, growth rate per annum [3]. To conserve the global wealth of medicinal plants and their biodiversity, cultivation is emerging as an economically viable option. It was states that in the USA alone, at least 118 of the top 150 prescription drugs are based on natural sources, including plants (74%), fungi (18%), bacteria (5%), and vertebrate species such as snakes and frogs (3%) [3, 5]. Many of these plants such as valerian (*Valeriana officinalis* L.) and fennel (*Foeniculum vulgare* L.) synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins [3,6,7]. Others contain alkaloids, glycosides, saponins, and many secondary metabolites, of which at least 12,000 have been isolated a number estimated to be less than 10% of the total [8-10]. Many of the herbs and spices used by humans to season food yield useful medicinal compounds [3,11-13].

Valerian (*V. officinalis* L.), a member of the Valerianaceae family, is a perennial herb that is

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widely used as a sleep aid. The dried rhizome and roots of valerian comprise the herbal drug valerian, which has been used for at least 1000 years [2,11]. The dried rhizome and roots of valerian is widely used as a mild sedative and sleep aid for insomnia, excitability, and exhaustion. Valerian has depressant activities equalising effects-acting as a sedative inagitated states and a stimulant in fatigue [12,13].

The modern agricultural activities are entirely based on use of inorganic fertilizers, therefore, fertilizer management is an important factor in quality and quantity crops yield. Although the applications of mineral fertilizers cause to increase the production, they are creating various problems on our environment and health [14-16]. Parallel to increasing the crop quantity by using the inorganic fertilizers, environmental protection as a sustainable agriculture is important today [17, 18, 19]. Thus, any operation and method that try to reduce the environmental pollutions will have a positive role in our life [20, 21]. The quality of medicinal plants is very important and can be influenced by choosing the right fertilizer to increases in secondary metabolites and antioxidant activity [8,22,23].

Bio-fertilizers such as nitrogen fixing, phosphate solubilizing and plant growth- promoting microorganisms along with some carrier material are able to provide a more nutrition for plants [24-26]. Unlike the chemical fertilizers which facilitate the absorb of only few minerals with impeding the uptake of others, bio-fertilizers can increase the deficient nutrients in the soil. Bio-fertilizers such as *Azotobacter chroococcum* and *Azospirillum lipoferum,* plays an important role in maintaining the productivity and sustainability of soil systems which are able to fix nitrogen with ability to release phytohormones to stimulate plant growth [27, 28, 29].

Various studies [30, 31, 32] have indicated that bio-fertilizer by increasing growth, essential oils and changing the chemical composition in medicinal plants play an important role to replace the inorganic fertilizers. Azimi *et al* [30] reported that yield and yield components of barley (*Hordeum vulgare* L.) increased after the application of nitrogen and phosphate biofertilizers. Also, similar results were obtained on application of Nitroxin biofertilizer on increasing the production quantity and quality of potato tubers compare with inorganic fertilizer [33-36].

Nitrogen and phosphor are among the major nutrients in plant nutrition and is a primary plant nutrient which nutrition influences both the primary and secondary metabolic pathways thus secondary plant metabolites accumulation [14,36].

Biofertilizers with have a positive effect on growth are more environmentally friendly and, in many cases, they have given the same or even better crop yields compared to mineral fertilizers [4,11,26]. Therefore, it is essential to combine the chemical fertilizers with some organic nutrients to increase the environment protection as well as improve the efficiency of chemical fertilizers.

The aim of this research was to evaluate the effects of urea as a chemical fertilizer and Nitroxin and Phosphate Barvar2 biofertilizers on yield and yield components of the valerian medicinal plants grown in the field.

MATERIAL AND METHODS

Experimental site

This study was conducted in Homad-Absard Agricultural Complex in Damavand city, Etka Organization, Tehran Province (45 km east Tehran, 35° 42’ N, 52° 20’E and an altitude of 2300 m), Iran in 2017-2020. The climate of the experimental according to the De Martonne classification was semi-arid with annual average temperature and rainfall were 10.5 °C and 320 mm, respectively. The soil was a clay loam that its characteristics were as follows: organic matter 0.35%, total nitrogen 0.03%, available phosphorus 16 ppm and available potassium 240 ppm and pH 7.2.

The experiment was set up as a factorial with 3 factors arranged in a randomized complete block design with three replications. Experimental factors consist Nitroxin (N0=control and N1=inoculation) and Phosphate Barvar2 (P0=control and P1=inoculation) biofertilizers each in 2 levels, and also the urea chemical fertilizer in 5 levels (U0=control, U1= 30, U2=60, U3=90 and U4=150 kg/ha).

Seeds of valerian were obtained from a commercial source, Pakan Seed, Isfahan, Iran. As the valerian seed, germinate very slowly which gives weeds and soil-borne pests more opportunity to damage the crop, a nursery bed was prepared in the greenhouse of the Agricultural college of Shahed University for raising seedlings, until they are eventually planted in a main plot. This will enhance the successful establishment of the young seedlings and make planting easier and provide a better environment for seeds to germinate and emerge, especially for small-seeded crops such as valerian. Before planting seeds, a nursery bed with a light and fluffy soil with good tithe was leveled with no clods or excess plant residue on the surface. Valerian Seeds were sown at greenhouse on January 15th 2017.

The field preparation was conducted during the months of September by a 30 cm depth plough to May followed by disc, rotivator and leveler. Each plot was 12 m^2 (4×3 m^2) with 6 rows of 60 cm inter row and 25 cm intra row spacing were prepared and treatments were allotted as per randomized plan. After 3 months, the healthy seedlings with about 15 cm tall and completely 4 true leaves and healthy roots were selected to transplant in the field. A 100 g and 1000 ml of Phosphate Barvar2 and Nitroxin biofertilizers were added in 5 L of water separately and mix thoroughly. On 5 April 2018, the valerian seedling roots were soaked with Nitroxin and Phosphate Barvar2 biofertilizers solution for about 10 min in the shade before culture. For combined application the treated valerian roots with Phosphate Barvar2 were soaked in Nitroxin solution after one hour and allowed to dry in the shade.

To measure valernic acid (%) root samples from the 24 months' plants were harvested and after clean and washed were freeze-dried for 2 days, at 10^{-1} mbar and -42 °C. The essential oils were extracted by hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil samples were to estimate the oil yields and percentage composition, after that were dried over anhydrous sodium sulphate and kept at −4 °C until it was analyzed. To distinguish valerenic acid from the complex matrix of the many other secondary metabolites present in *V. officinalis* of GC-MS (Gas chromatography-mass spectrometry), Thermo-Ultera Fast Model, Italy was used.

Valerian plants were harvested 21 months after transplanting. Measured parameters were root diameter (mm), root length (cm), root dry weight (kg/ha), shoot dry weight (kg/ha), leaf width (cm), leaf length (cm), valernic acid (%) and valernic acid yield (g/ha). Analysis of variance (ANOVA) was conducted using SAS software [37] with the differences between treatments was determined by the test of Least Significant Difference (L.S.D.).

RESULTS

The analysis of variance of this experiment showed that most of measured parameters such as valernic acid (%), valernic acid (g/ha), shoot and root dry weight (kg/ha), leaf width (cm), leaf length (cm) and root length (cm) in valerian plants were significantly ($p \leq 0.01$) affected by the application of various amounts of urea and biofertilizers (Table 1). Except the valernic acid (%), all the measured traits were uniformly improved by increasing the urea fertilizer (Table 1).

Valernic acid: Both valerinic acid percentage and yield were significantly ($p \leq 0.01$) affected by the application of various amounts of organic and inorganic fertilizers (Table 2). Results showed that the effect of urea, Nitroxin and Phosphate Barvar2 biofertilizers on the valerinic acid (%) compare to control were significant ($p \leq 0.01$). The highest valernic acid (%) obtained for control (0.465%), compared to 39, 38, 24, 39 and 54% reduction at 150 kg/ha urea, Nitroxin, and Phosphate Barvar2, interaction of Nitroxin, and Phosphate Barvar2 and combination of 150 kg/ha urea, Nitroxin, and Phosphate Barvar2 application treatments, respectively (Table 2). Results showed that the maximum (1490 g/ha) and minimum (712 g/ha) valernic acid yield was recorded by the combination of Nitroxin and Phosphate Barvar2, 150 kg urea/ha and control, respectively (Table 2).

Root dry weight (kg/ha): Experimental results showed that urea, Nitroxin, Phosphate Barvar2 fertilizers and interaction between them had significant influence on root dry weight (Table 1). The application inorganic and organic fertilizers to valerian led to significant increase of the vegetative growth characters and yield components with corresponding increase in the major component content of essential oil. There were 61, 58, 48 and 68% increases in root dry weight at 150 kg ha urea, Nitroxin, Phosphate Barvar2 fertilizers and interaction between them (Nitroxin, Phosphate Barvar2, 150 kg/ha urea) treatments respectively compare to control (Table 2). Urea at the combination of Nitroxin, Phosphate Barvar2, 150 kg/ha urea had its best effects on root dry weight (665 kg/ha) compared to the control (215 kg/ha).

Shoot dry weight (kg/ha): A significant increase was observed in shoot dry weight after the Phosphate Barvar2, Nitroxin and urea application (Table 2).

Urea at 150 kg/ha, Nitroxin, Phosphate Barvar2 and interaction between them (Nitroxin, Phosphate Barvar2 and urea at 150 kg/ha) significantly increased shoot dry weight by 57.2, 54, 52 and 68% respectively (Table 2).

Table 1 Analysis of variance for morphological traits and phytochemical properties of valerian under different levels of urea, Nitroxin and Phosphate Barvar2 fertilizers

SOV	Df	(MS							
		Root diameter	Root length	Root dry weight	Shoot dry weight	Leaf width	Leaf length	Valernic acid (%)	Valernic acid yield
Urea (U)	4	0.183 ns	4.020 *	49219.63 **	475628.03 **	0.083 *	110.40 **	194423 **	194423 **
Nitroxin N)	1	0.260 *	18.144 *	151237.50 **	22881.23 **	0.355 *	70.62 **	445321 *	445321 *
P Barvar2(PB)	1	0.245 *	17.340 *	221313.78 **	20653.48 **	0.311 *	64.64 **	372610 *	372610 *
U×N	4	0.385 **	2.55 ns	16710 *	36512.47 **	0.08 *	15.88 **	56613 *	74036 *
U×P B	4	0.296 **	3.070 ns	15321.55 *	51798.23 **	0.01 *	14.31 **	63252 *	69517 *
N×P B	1	0.217 **	2.190 ns	14474 *	47305.11 **	0.007 *	13.23 **	77355 *	70341 *
U×N ×P B	4	0.430 **	2.770 *	13421.42 *	54909.65 **	0.009 *	16. 33 **	83523 *	83523 *
Replication	2	0.054	3.221	3055.63	3424.41	1.344	18.18	19044	19044
Error	38	0.097	2.030	*3975.11*	*3182.26*	0.188	9.219	39144	39144
(CV%)		17.44	6.56	22.30	21.32	10.11	16.43	10.10	23.00

*, **: significant at P= 0.05 and 0.01 levels, respectively.

Table 2 Means comparison of yield and yield components of valerian under different levels of urea, Nitroxin and Phosphate Barvar2 fertilizers

Nitroxin	P-Barvar 2	Urea	Root diameter (mm)	Root length (cm)	Root dry weight (kg/ha)	Shoot dry weight (kg/ha)	Leaf width (cm)	Leaf length (cm)	Valernic Acid (%)	Valernic Acid yield (g/ha)
		U0	1.60 e	16.05 e	215 e	201 d	4.05 c	15 e	0.463 a	712 e
		U1	1.75 d	17.85 d	345 d	245 c	4.150 c	18.10 d	0.432 b	760 d
	P0	U2	1.95 c	20.90 c	410 c	308 b	4.35 b	22.20	0.381 c	870 c
		U3	2.23 b	22.35 b	470 b	455 a	4.55 a	23 b	0.335 d	1020 b
N0		U4	2.40 a	24.00 a	550 a	470 a	4.60 a	24 a	0.285 e	1150 a
		U0	1.89 d	18.85 d	420 d	420 d	4.14 c	21 e	0.335 a	890 d
		U1	2.03 c	19.42 c	419 d	429 d	4.15 bc	22 d	0.350 ab	903.33 d
	P1	U2	2.13 bc	19.58 c	460 c	473 c	4.25 b	23 c	0.330 b	951.66 c
		U3	2.22 ab	20.25 b	520 b	540 b	4.27 a	24 b	0.235 c	1160 b
		U4	2.33 a	22.37 a	625 a	590 a	4.30 a	25 a	0.230 c	1395 a
		U0	1.99 d	16.23 c	505 d	435 e	4.35 b	24 c	0.290 a	1123 d
		U1	2.13 c	20.04 b	515 d	460 d	4.43 b	24 c	0.238 b	1080 e
	P0	U2	2.17 bc	20.17 b	545 c	493 c	4.43 ab	24 c	0.233 b	1175 c
N1		U3	2.26 ab	20.27 b	587 b	550 b	4.45 ab	24 b	0.230 b	1261 b
		U4	2.37 a	23.47 a	640 a	625 a	4.47 a	25 a	0.225 b	1440 a
		U0	2.08 c	19.25 c	535 d	490 d	4.40 b	25 d	0.303 a	1100 d
		U1	2.14 c	20.15 b	545 d	505 d	4.45 ab	26 c	0.296 a	1144 c
	P1	U2	2.19 bc	20.23 b	570 c	535 c	4.48 ab	26.15 bc	0.290 a	1235 b
		U3	2.30 ab	20.29 b	600 b	535 b	4.49 ab	26.35 ab	0.285 a	1160 c
		U4	2.39 a	23.59 a	662 a	635 a	4.51 a	26.75 a	0.215 b	1490 a

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using LSD Test.

Root length (cm): There was significant increase in root length with urea, Nitroxin, Phosphate Barvar2 fertilizers and interaction between them compare to control (Table 1). Root length (cm) mean in urea at 150 kg/ha, Nitroxin, Phosphate Barvar2 fertilizers treatments and interaction between them were 24, 19.40, 18.88 and 23.59 cm compared to 16.05 cm in the control respectively (Table 2). The results suggest that urea fertilizer concentration had a significant effect on the valerian root length compare to control (Table 2).

Leaf length and width (cm): The results indicated that urea at 150 kg/ha, Nitroxin biofertilizer and interaction between them (150kg/ha urea×Nitroxin) were significantly increased (37.08. 51.2, and 40.15%, respectively) the leaf length (cm). Despite 20.50% increase in Phosphate Barvar2 application, no significant differences in leaf length were observed compared to untreated plants (Table 2).

Analysis of variance of the results showed significant differences in leaf width due to application of urea at 150 kg/ha and Nitroxin fertilizers. Significantly increases with the valerian leaf width relative to control under urea, Nitroxin, fertilizers and interaction between them (150kg ha/urea × Nitroxin) treatments were 12, 7 and 10% respectively. Phosphate Barvar2 application had no significant effect on the leaf width, although an increase (2.17%) in leaf width was observed.

DISCUSSION

This experiment illustrates the importance of understanding the alternative effects of biofertilizers to enhancing the growth attributes of valerian. Biofertilizers appear to be the major substitute factor to inorganic fertilizer for valerian and/or other medicinal plants [38,39]. Biofertilizers by increasing the abundance of soil organisms make a positive effect on yield and yield components of many crops. The mineral N and P fertilizers can be replaced by biofertilizers, which can reduce both the production costs and the damages to the environment. Nitroxin and Phosphate barvar2 biofertilizers consists the most effective species of nitrogen and Phosphate stabilizing bacteria for increase efficiency of crop production [40-43]. In addition, these results agree with those of Abou-Zeid *et al* [41] and Azimi *et al* [30] who all found the significant influence of the application of Nitroxin and Phosphate Barvar2 biofertilizers on different crop yield. The results were in agreement with those proposed by Abdi *et al* [42] who found that Nitroxin and P barvar2 biofertilizers were effective in increasing the traits measured of valerian. This experiment agrees with those of Adegbidi *et al* [36], Damnjanović *et al* [34] and Zheljazkov and Warman [43] who concluded that different fertilizer levels are able to decline the essential oil composition of valerian roots.

This finding was similar to those of study of Basil (*Ocimum basilicum* L.) by Rahimi Shokooh *et al* [18] who concluded that biofertilizers have its ability to increase the availability of phosphorus and other nutrients.

Mikovacki and Milic [44] and Chand *et al* [45] observed that Nitroxin biofertilizer had significant effects on root length. They concluded that under the effect of single and combined application of N and P biofertilizers which induced the uptake ability of the roots to nutrients and positive increase in the yield parameters because of improving the root system.

This study suggests that providing a biofertilizer contact time would be crucial for the success of a medicinal plant growth. Penzkofer *et al* [11] stated that the using of Nitroxin and Phosphate Barvar2 biofertilizers improved growth and increased shoot dry weight. They recommended treatment rate of biofertilizer for growth advantage of medicinal plants in a minimum contact time of 10 minutes. These results are in agreement with Dauda *et al* [12] and Filizadeh and Goodarzi [46] who found that the maximum biomass in target plants was observed under application of biofertilizers, which positively influenced the plant photosynthesis and dry matter accumulation. They also suggest that with an increase store carbohydrate after of inoculation with biofertilizers, the plant will able to respond positively in increased growth. Similar results were obtained by Khanna *et al* [38] on valerian who reported that leaf length and width by applying the NPK along with biofertilizers increased.

The findings of the present study suggest that Nitroxin and Phosphate Barvar 2 biofertilizers have positive effect on yield and yield components of valerian due to synergistic effect of them. Yehya and Mohamed [4] in agreement with results of this experiment found that, combined application of N and P biofertilizer with inorganic fertilizer has a better effect on yield and yield components of target plants than single application of them. Therefore, combined application of biofertilizers with inorganic fertilizers had more efficiency than single application due to some positive interaction between their microorganisms and finally laid to increase in yield components [47-54]. Azzaz *et al* [55], Bhatt *et al* [56] and Enwall *et al* [57] in agreement with this experiment provided evidence that combined fertilizer was better medicinal plants like valerian than single application of fertilizer.

CONCLUSIONS

This study serves as a confirmation towards the important role of biofertilizers in increasing the yield of various crops. Also, the present study demonstrates that focusing on the biofertilizers is an effective way to replace the inorganic fertilizers, although an extensive review on growth on response of valerian and other medicinal plants to different biofertilizers is necessary for further research. Also results show that application of Nitroxin and Phosphate Barvar2 biofertilizers compared to urea inorganic fertilizer had a better effect on valernic acid percent. In addition, according to the results it can be concluded that the combination of organic and inorganic fertilizers causes a reduction of the chemical fertilizer consumption to half. This would improve the growth and quality of valerian medicinal plants and preserve the environment.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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