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Investigation of Phosphorus and Potassium Biological Fertilizers on Alfalfa (*Medicago sativa* L.) Physiological Traits at Different Ages of the Field

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ABSTRACT

Background: Alfalfa (*Medicago sativa* L.) is one of the most productive plant in terms of nitrogen fixation and the suitable amount of protein and carotene has a significant impact on the nutritional quality of alfalfa.

Methods: This combined experiment was conducted as factorial based on a randomized complete block design with three replications during two consecutive years (2019-2020) under the climatic conditions of Jiroft area (Kerman province, Iran). The studied factors included alfalfa age (one, three and five-years old) and bio-fertilizers (Control, Potash-Barvar (KSB), Phosphate Barvar II (PSB), Potash-Barvar + Phosphate Barvar II) (KSB+PSB). The biofertilizers were used along with irrigation water. The studied physiological traits included nitrogen, phosphorus, crude protein, soluble sugars, malondialdehyde (MDA) and sodium amount.

Result: The highest amount of crude protein, nitrogen, phosphorus and soluble sugars was observed in three-years old fields under the treatment of bio-fertilizers Potash Barvar + Phosphate Barvar II (KSB+PSB) (37.78%, 9.79%, 0.51% and 324.75% respectively). Unlike other treatments, the highest amount of sodium (0.95%) and malondialdehyde (MDA) (83.99 μM $^{g-1}$FW) was measured for alfalfa in treatment one-years old field under no-fertilizers (control), while the lowest amount of sodium (0.49%) and malondialdehyde (MDA) (55.94 μM g^{-1}FW) was observed in treatment five-years old field under bio-fertilizers Potash Barvar + Phosphate Barvar II (KSB+PSB) application.

Key words: Alfalfa physiological traits, Phosphorus biological fertilizer, Potash biological fertilizer.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a plant that has stolone, roots are strong, well developed that need plenty of oxygen and adequate moisture and the first established plants in crop rotation. The green organs this plant, like leaves, contain a substance called saponin, which causes bloat in animals. Jiroft area and the south of Kerman province, Iran with an area under cultivation of about 12000 hectares, is one of the most prone areas for cultivating this crop in the southeast of Iran. On the other hand, in plant nutrition, phosphorus is one of the most consumed nutrients and its deficiency in plants is the second major problem of soil fertility worldwide. Alfalfa also needs high levels of potassium to produce optimal yield. Alfalfa requires potassium to catalyze several metabolic actions. Functions such as enzyme activation, transpiration, displacement of compounds made in the optical process, protein and starch synthesis and energy relationships all require potassium Bernardi *et al.* (2013). Studies have also been conducted in this regard. Kumar *et al.* (2015) reported that application of azospirillum with 93.75 kg/ha of nitrogen and phosphorus in *Artemisia pallens* L. increases growth, fresh and dry biomass.

The results of a study conducted by Ratti *et al.* (2001) indicated that the combination of mycorrhizal fungus with plant growth promoting bacteria such as Bacillus and Azospirillum led to an increase in biomass and phosphorus contents of Hypericum perforatum. A research conducted by Crespo *et al.* (2011) showed that phosphate-solubilizing bacteria are a very good alternative to phosphate fertilizers. Studied the effects of drought stress and phosphorus on grass pea (*Lathyrus sativus* L.), the use of phosphorus fertilizer (60 kg/ha) increased the yield of grass pea (2401 kg/ha). This means that phosphorus fertilizer can partially compensate for the effect of drought stress and highly impacts water use and phosphorus agronomic efficiency Bahramnejad *et al.* (2021).

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Oral *et al.* (2021) investigated the effects of rhizobacteria and blue-green algae on some physiological characteristics of soybean *Glycine max* L. cultivated under water stress that rhizobacteria and blue-green algae have a reducing and regulating effect on the investigated physiological properties.

Yannan *et al.* (2022) concluded that legumes are low-cost but high-yield products that are rich in proteins, vitamins and minerals. Legumes, known as mycorrhizal plants, are widely used as model organisms to investigate plant-microbe interactions, especially the symbiotic relationship between plants and rhizosphere microorganisms. Arbuscular Mycorrhizal Fungi (AMF), an important class of plant-associated microbes, can regulate many physiological and molecular responses of plants.

In a study on improving the quantitative and qualitative yield of alfalfa with the application of chemical and biological fertilizers, Madani *et al.* (2015) showed that the highest yields of dry alfalfa was obtained in the first harvest at 1.76 tons per hectare under the application of phosphorus-releasing bacteria and at 1.57 tons per hectare with application of potash solubilizing bacteria. Given what was stated above, the study was conducted to investigate the relationship between field age and the application of phosphorus and potash biological fertilizers on physiological traits of alfalfa under climatic conditions of Jirort area.

MATERIALS AND METHODS

Experimental design

In order to study the effect of the use of phosphorus and potassium biological fertilizers on the physiological characteristics of alfalfa variety Nikshahri at different ages, a factorial experiment based on a randomized complete block design with three replications and during two crop years (2019-2020) in Jiroft area (Kerman province, Iran)was done. Climatic conditions and edaphic characteristics of the research area are presented in (Fig 1 and Table 1), respectively. The studied factors include year (two consecutive years), three ages of alfalfa (one, three and five-years old) and four levels of biological fertilizer (control, Potash-Barvar (KSB)at the rate of 100 grams per hectare, Phosphate Barvar II (PSB) at the rate of 100 grams and the application of Potash-Barvar + Phosphate Barvar II) (KSB+PSB) was 100 grams per hectare. In order to create the same climatic and edaphic conditions and reduce the error of the experiment, the selected field was selected in the first year, with a larger area and different ages of alfalfa and every two years, the design was carried out in the same field.

In other words, to provide the same levels of alfalfa age compared to the first year, other blocks in the same field were used. The studied biological fertilizers were used as a solution in irrigation water. Phosphate Barvar II fertilizer contains two types of phosphate-dissolving bacteria, including *Panteoa agglomerans* and *Psuedomonas putida* and Potash Barvar biological fertilizer contains two types of potassium-dissolving bacteria, *Pseudomonas koreensis* strain S14 and *P. vancouverensis* strain S19. In each year, 36 experimental plots (3 field ages × 4 levels of biological fertilizer × 3 replications) with dimensions of (6 × 2) square meters were considered. The best time to plant alfalfa (cv. 'Nikshahri') is from mid-March to mid-April when the danger of winter cold is removed, or from early October to early November when the summer heat subsides.

Measurements

The studied physiological traits included nitrogen, phosphorus, protein, soluble sugars, malondialdehyde (MDA) and sodium. Soluble sugars were measured by the Kochert method (Kochert, 1978). For this purpose, 0.1 gram of plant tissue and root was mixed with 10 ml of 80% ethanol after drying in an oven at 70°C for one week. After one week, the supernatant solution was used to measure the amount of soluble sugars. The amount of nitrogen in the plant was measured by the Bremer method (Bremer, 1960). Sodium and phosphorus elements were measured by the soil laboratory. The amount of sodium in leaves was used according to the method provided by (Hamada and Elnay, 1994). The amount of phosphorus was also measured by

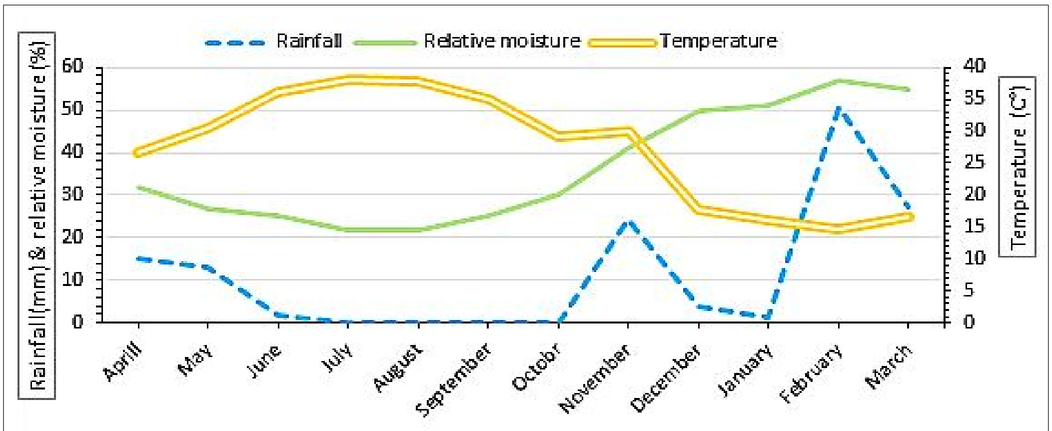

Fig 1: Mean temperature, rainfall, relative moisture at experimental site (Jiroft, Kerman) during 2019-2020.

reading the absorbance of the digested extract containing nitrovana domolybdate reagent at a wavelength of 430 nm using a spectrophotometer. The amount of protein was measured using the conversion factor and the product of nitrogen percentage in a fixed number of 6.25 McDonald *et al.,* (1991). The malondialdehyde (MDA) was measured using a modified thiobarbituric acid method Yan *et al.* (2022).

Statistical analysis

Before analysis of data, the normality was assessed using SPSS software and Kolmogorov-Smirnov and Shapiro-Wilk methods. Normal data were analyzed using SAS software and Duncan's multiple range test, the means were compared at the probability level of 1 and 5%.

RESULTS AND DISCUSSION

Comparison of the mean square physiological traits evaluated in alfalfa under the influence of simple effects of year, life of alfalfa and biological fertilizer showed that the amount of nitrogen, phosphorus, protein, soluble sugars in three-years old and five-years old alfalfa field significantly higher than in one-year old field (9.79%, 0.51%, 37.78% and 324.75 mg.L^{-1} respectively) while the highest amount of sodium (0.95%) and malondialdehyde (MDA) (83.99 µM g^{-1}FW) was measured for alfalfa in treatment one-years old field under no-fertilizers (control), while the lowest amount of sodium (0.49%) and malondialdehyde (MDA) (55.94 µM g^{-1}FW) was observed in treatment five-years old field under bio-fertilizers Potash Barvar + Phosphate Barvar II (KSB+PSB) application (Table 2). Organic production systems seek to improve soil organic matter and biological diversity, which may impact P cycling and P uptake by crops. Phosphorus fertilization in the organic production system entails balancing the P inputs with crop removal by selecting and managing both nitrogen and P inputs (Khangarot *et al.* 2022). Phosphate solubilizing bacteria (PSB) are beneficial bacteria accomplishing in solubilizing inorganic phosphorus from insoluble compounds Chen *et al.,* (2006). The mechanism of mineral phosphate solubilization by PSB strains is associated with the release of organic acids in the soil. These low molecular weight organic acids have hydroxyl and carboxyl groups that can chelate the cations bound to phosphate, resulting in the conversion of insoluble Phosphorus to its soluble forms. The collaboration of PSB and pulses enhances soil fertility and is a cost effective way of phosphate fertilization in legumes. Certain phosphate solubilizing bacteria acts as Plant Growth Promoting Rhizobacteria (PGPR) *i.e.* one of the classes of beneficial bacteria residing in the rhizosphere Kloepper *et al.* (1989). *Pseudomonas* fluorescens (PF) is a gram-negative bacterium that colonizes roots of agricultural crops; provide essential services to the agro-ecosystem as they encourage plant growth and health by overpowering soil-borne diseases, by stimulating plant immune defences and by improving nutrient accessibility in soil. *Pseudomonas* fluorescens has the capacity to mobilize inorganic phosphate in agricultural soils (Browne *et al.*, 2009). It solubilizes about 30 per cent of soil phosphorus. Khangarot *et al.,* (2022) reported that application of PROM+PSB+VAM+PF was found the most superior treatment combination for obtaining higher values of growth, yield and nutrient uptake in mungbean.

The studies conducted by (Fan *et al.,* 2011; Jia *et al.,* 2006 and Zhou *et al.,* 2015) showed that the content of soil nutrients including organic matter, N and P improved in fertilized soil compared to non-fertilized soil. The increased

Table 1: Physico-chemical properties of experimental soil before initiation of field experimentation.

Soil parameters	Status/Value 2019		Status/Value 2020		Methods employed
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	International pipette method
Mechanical separates (%)	Deep 0-30 cm	Deep 0-60 cm	Deep 0-30 cm	Deep 0-60 cm	(Piper, 1950)
Sand	76	75	77	76	
Silt	11	11	12	12	
Clay	13	14	13	12	
Chemical properties					
Soil reaction (pH)	8	8.1	8.3	8.5	1:2.5 soil:water suspension
Organic carbon (%)	0.36	0.19	0.47	0.44	(Jackson, 1967)
EC(ds /m)	0.48	0.39	0.56	0.41	Rapid titration method (Walkley and Black, 1934)
Available nutrients(mg kg^{-1})					
• P	8	3.9	8.4	4.2	DTPA method (Lindsay and
• K	270	193	223	182	Norvell, 1978)
DTPA extractable micronutrients (mg kg^{-1})					
• Fe	4.4	3.2	4.1	3.3	
• Mn	5.6	4.5	5.1	4.2	
• Zn	3.3	2.1	3	2	
• Cu	1.3	0.9	1.1	0.8	

Table 2: Comparison of the means of physiological traits evaluated in alfalfa under the effect of alfalfa age and biofertilizer during 2019-20. (pooled data of 2 years).

Field age	Fertilizer treatment	Crude protein (%)	Nitrogen (%)	Phosphorus (%)	Sodium (%)	Malone dialdehyde (MDA) (μM g^{-1}FW)	Soluble sugars (mg.L^{-1})
One-years old	Fertilizer control	16.68d	3.81g	0.24g	**0.95a**	**83.99a**	221.33f
One-years old	P_2O_5 (PSB)[1]	20.40cd	5.19ef	0.44cd	0.84b	70.72b	247.24de
One-years old	K_2O (KSB)[2]	21.29cd	6.05d	0.37e	0.77cd	62.92c	245.53de
One-years old	P_2O_5+ K_2O (PSB+KSB)	25.70bcd	7.29c	0.42d	0.73de	51.76d	266.64cd
Three-years old	Fertilizer control	18.99cd	5.00ef	0.27f	0.82bc	78.16a	235.92ef
Three-years old	P_2O_5 (PSB)	21.92cd	6.78c	0.46bc	0.69e	64.06c	261.64de
Three-years old	K_2O (KSB)	32.49ab	9.04b	0.41d	0.68e	62.29c	301.84b
Three-years old	P_2O_5+ K_2O (PSB+KSB)	**37.78a**	**9.79a**	**0.51a**	0.55f	55.29d	**324.75a**
Five-years old	Fertilizer control	17.28d	4.66f	0.27f	0.67e	83.08a	234.07ef
Five-years old	P_2O_5 (PSB)	21.37cd	5.62de	0.44cd	0.54fg	66.54bc	244.67def
Five-years old	K_2O (KSB)	23.28cd	7.20c	0.42d	0.60f	63.24c	272.81c
Five-years old	P_2O_5+ K_2O (PSB+KSB)	27.59bc	8.77b	0.48ab	0.49g	55.94d	296.22b
	Mean square	57.78**	1.25**	8.10**	0.10**	38.49**	1280.51**
	LSR 1%	18.45	1.37	0.35	0.45	12.76	48.64
	CV(%)	29.45	7.94	6.50	6.25	7.26	6.97

Note: 1. Phosphorus biological fertilizers.

2. Potash biological fertilizers.

3. Mean within columns followed by different letter are significantly different (Duncan's multiple test, P≤0.05).

4. n.s: non-significant; *: Significant at 5% probability levels and **: Significant at 1% probability levels, respectively.

availability of nitrogen and phosphorus in the root zone coupled with improved metabolic activity at cellular level might have increased the nutrient uptake and their accumulation in the vegetative plant parts (Dutta *et al.* 2021). Karayilanli and Ayhan, (2015) reported P content of alfalfa decreased with maturity.

The increased availability of nitrogen and Phosphorus in the root zone coupled with improved metabolic activity at cellular level might have increased the nutrient uptake and their accumulation in the vegetative plant parts (Dutta *et al.,* 2021 and Kumar *et al.,* 2021). Increased accumulation of nutrients in vegetative parts of the plant with improved metabolism led to greater translocation of these nutrients to reproductive organs of crop and ultimately the nutrient contents of seed and straw of crop plant enhanced at harvest. Significantly higher N, P and K uptake is directly associated with the higher nutrient content in seed and straw and higher grain and straw yield obtained under these superior treatments (Baljeet *et al.,* 2020 and Kumar *et al.,* 2018). The results have evidently proved the advantage of combining The combination of biological fertilizers improves some physiological traits of the alfalfa plant.

CONCLUSION

According to the results of this research, it was determined that the use of biological fertilizers, especially the combined use of Potash Barvar + Phosphate Barvar II (KSB+PSB) each at the rate of 100 grams per hectare improved the physiological characteristics of alfalfa, especially in older fields. Based on this, the combined use of potassium and phosphorus biological fertilizers can be an efficient solution to reduce the loss of alfalfa physiological characteristics in old age.

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Conflict of interest: None.

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