

Agro-physiologic Traits of Soybean in Response to Biological Fertilizers

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ABSTRACT

Plant nutrition is one of the most important environmental factors affecting the quantitative and qualitative yield of crops. This study investigated the effect of rhizobium and azospirillum inoculum and foliar application of humic acid and nutrients of molybdenum, cobalt, and boron on node formation, yield, yield components, and morpho-phenological traits of soybean (Saman cultivar). The experiment was carried out in a factorial randomized complete block design at Golestan Agricultural and Natural Resources Research Center in 2018 and 2019 in three replications. The results showed that experimental treatments had a significant effect on the measured traits. Most of the measured traits, including yield, under the influence of seed treatment with bacteria azospirillum and cobalt, molybdenum, boron showed more increase than the control. It was found that under humic treatment, application of bacteria and nutrients can be effective on improvement of seed yield, so the highest seed yield mean (3268 kg/ha) was obtained by humic acid + Rhizobium+Azospirillum + Co+Mo+B treatments. Also, the highest protein yield mean was obtained by humic acid + Rhizobium + Co+Mo+B treatments (1103 kg/ha) and highest mean of oil yield was obtained by humic acid + Rhizobium+Azospirillum + Co+Mo treatments (577 kg/ha). Overall, the results of this study confirmed the efficiency of nitrogen-fixing bacteria as well as foliar application of micronutrients in increasing soybean yield per unit area.

Keywords: *Azospirillum*, foliar application, micronutrients, *Rhizobium*, soybean.

INTRODUCTION

Soybean (*Glycine max*) is one of the most important oil-protein plants belonging to the Fabaceae family whose seeds contain approximately 20% oil and 40% protein and is considered the most important source of vegetable oil and protein (Sinclair, 2017; Bagale, 2021). Excessive use of chemical fertilizers in recent years has raised concerns in agriculture, environment, industry, and health, and groundwater pollution with nitrate is one of the major environmental challenges in the world (Rahman and Zhang, 2018; Tyagi et al., 2022). Considering the annual consumption of chemical fertilizers in the lands under cultivation of dark legumes, it is necessary to use inoculants effectively for each of the agricultural legumes of the country, with proper planning. Soybeans should be produced and provided to farmers (Igbal et al., 2022) Micronutrients such as boron are essential for plant growth and

development. After iron and zinc, boron deficiency (less than 15 ppm) causes the most damage to plants (Brdar-Jokanović, 2020).

Biological fertilizers or microbial fertilizers (usually in solid, liquid, and semi-solid forms) contain substances with one or more specific species of microorganisms and contribute to the expansion of the root system and absorption of elements, inducing plant growth and enhancing yield by increasing the morphological and physiological characteristics of plants (Egamberdiveva, 2007; Zhang et al., 2022). Humic acid is a nature-friendly organic fertilizer that due to the presence of hormonal compounds, even very small amounts of it have beneficial effects on increasing and improving the production of agricultural products. Humic acid is extracted from various sources such as soil, humus, peat, oxidized lignite, coal, etc. (Nasiri Dehsorkhi et al., 2018).

Implementation of integrated plant nutrition using bacterial biofertilizers

(Azotobacter, Azospirillum, and Pseudomonas) along with chemical fertilizers has led to increased vegetative growth and improved reproductive growth, which in turn increased growth and development (de Borja Reis et al., 2022). Dabaghian et al. (2015) examined the effect of Azotobacter, Azospirillum, and organic sulfur biofertilizers on soybean production and yield and found that the addition of sulfur increases the number of nodes, root dry weight, and plant height in soybeans. Moreover, a significant difference in the yield of this plant was caused by the use of Azotobacter and Azospirillum bacteria.

Rose et al. (2002) reported interesting results by foliar application of different concentrations of boron in two stages: V3 (emergence of the first three-leafed leaf) and R2 (end of flowering and beginning of seedling). Thus, application of 1 to 2 kg of boron per hectare produced the highest concentration of boron in seeds (21-27 mg boron per kg of seed) and application of boron in stage R2 significantly increased boron compared to V3 in seeds. Boron application time had a significant effect on germination rate and seed weight so that in the absence of boron consumption, the lowest seed germination rate was 60% and in boron consumption, the highest seed germination rate was 96% (consumption in stage V3) and 94% (consumption in step R2). Consumption of boron in stage V3 increased the weight of seeds from 12.2 to 12.8 grams and consumption in stage R2 increased the weight of seeds from 13.7 to 14.8 grams. The authors also stated that boron concentration is a good indicator to determine boron deficiency in post-harvest diagnoses.

Ross et al. (2006) examined different proportions of boron in soybeans and concluded that the application of boron in three of the four study areas increased seed yield. The use of boron also increased the concentration of boron in tricolor leaves and

soybean seeds and stated that high concentrations of boron in seeds could increase the resistance of seedlings to boron deficiency in the early stages of growth. Bellaloui et al. (2010) reported that foliar application of boron increases the activity of nitrogenase and nitrate reductase in leaves, highlighting the important role of boron in nitrogen metabolism. If the concentration of boron in the leaves is low, it is not possible to transfer boron from mature and mature tissues to young tissues and seeds. Moreover, the consumption of boron in leaves increases protein and oleic acid in seeds and decreases fat and linoleic in seeds. Increasing protein and oleic acid has come at the cost of reducing fat and linoleic acid.

The main purpose of this study was to investigate the effect of rhizobium, azospirillum, humic acid, molybdenum, cobalt, and boron on nodule formation and agro-morphological traits of soybeans.

MATERIAL AND METHODS

In order to investigate the effect of rhizobium and azospirillum inoculum and foliar application of humic acid and nutrients of molybdenum, cobalt, and boron was examined on nodule formation, yield, yield components, and phenological and morphophysiological traits of unlimited and late growth of soybean cultivar under summer cultivation conditions, an experiment was conducted based on randomized complete blocks in Gorgan Agricultural Research Station in 2018 and 2019. The experimental factors include foliar application of humic acid (consumption of folic acid with folic acid in two stages V5, V3; and control), bacterial seed (rhizobium, rhizobium + azospirillum, and control), and foliar application of nutrients (cobalt; cobalt + molybdenum; cobalt + molybdenum + boron; and control).

Table 1. Maximum, minimum, and mean monthly air temperature, rainfall, and reference evapotranspiration (ET₀) in 2018-2019 and 2019-2020

Year	Month	T _{min} (°C)	T _{max} (°C)	T _{mean} (°C)	Rain (mm)
2018	July	19.9	43.5	31.0	23.4
	August	20.1	39.5	30.2	21.3
	September	14.5	37.3	26.8	09.2
	October	07.3	33.4	20.8	54.5
	November	04.7	39.6	15.4	47.3
	December	01.4	26.1	11.5	43.8
2019	July	17.9	39.1	29.0	24.7
	August	15.2	41.9	28.2	04.2
	September	16.8	38.4	25.6	22.6
	October	08.2	35.5	21.8	62.2
	November	03.7	26.9	14.4	106.1
	December	-01.3	22.5	10.0	08.1

Foliar application of cobalt and molybdenum was performed in two stages V5, V3, and boron foliar application in two stages R3, R1 at the recommended dose for each compound. Experimental planting was done in early July each year. Each experimental plot consisted of 6 planting lines 5 m long with a row spacing of 50 cm

and a row spacing of 15 cm. The distance between the plots was not one row and the distance between the two replicates was 2.5 m. Irrigation was done using type strips based on 80 mm evaporation from Class A evaporation pan and the application of basic fertilizers according to soil test results (Table 2).

Table 2. Physiochemical characteristics of the soil of the experimental field

Soil characteristics	Depth (cm)			
	0-15	15-30	30-60	60-90
pH	2.7	3.7	3.7	3.7
EC (dSm ⁻¹)	35.1	27.1	42.1	41.1
Organic carbon (%)	15	1.1	6.0	4.0
Total nitrogen (%)	15.0	11.0	06.0	03.0
Available Phosphor (ppm)	6.8	8.4	2	01.1
Available potassium (ppm)	333	220	108	70
Bulk density (g.cm ⁻³)	44.1	41.1	4.1	4.1
Soil texture				
Clay (%)	28	30	34	33
Silt (%)	54	52	52	52
Sand (%)	18	18	14	15
Soil texture	Loam-Silty	Loam-Silty	Silty-Clam-Loam	Silty-Clam-Loam
Water content				
Saturation point (%) (θ _m)	9.49	2.52	9.51	60
Field capacity (%) (θ _m)	7.27	27	27.6	27.7
Permanent wilting point (%) (θ _m)	1.13	12.3	9.8	9.8

The grain yield (Y) and morpho-phenological characteristics were obtained directly using scales and rulers and direct counting of samples. The traits include day to maturity (DM), plant height (PH), 100-Seed Weight (SW), biological yield (BY), harvest index (HI), protein content (PC), oil content (OC), protein yield (PY), and oil yield (OY).

Statistical analysis

The collected data were analyzed using SAS 9.1 statistical software and the means were compared using LSD test at 5% probability level and graphs were drawn with

Excel software (version 2019).

RESULTS AND DISCUSSION

Soybean is a crop which is affected by agronomical management. In the present study, some nitrogen sources and micronutrients applications were assessed in soybean during two cropping seasons. The results showed that humic acid, rhizobium and azospirillum inoculation, as well as micronutrient fertilizer treatments had significant effects on the surveyed traits except for biological yield (Table 3).

Table 3. Analysis of variance for the surveyed traits in soybean

S.O.V.	df	PH	DM	SW	BY	Y	HI	PC	OC	PY	OY
Year (Y)	1	2847.11**	823.69**	12.25**	17461255*	6990031**	353*	54.85**	75.75**	826795**	161123**
Block (Year)	4	97.67	4.77	3.54	2374776	112739	120	9.28	9.27	12718	2322
Humic acid (a)	1	1125.04*	142.80**	84.03**	3002134 ^{ns}	42733023**	9940**	64.95**	227.71**	5132632**	1450738**
Bacteria (b)	2	1262.49**	99.61**	66.50**	485717 ^{ns}	1065707**	237*	42.06**	44.80**	112403**	65284**
Micronutrient (c)	3	1747.21**	118.19**	22.80**	430492 ^{ns}	2116901**	423**	13.39**	19.22**	218663**	61838**
a*b	2	917.45**	30.14**	2.87**	930225 ^{ns}	415703**	92.8 ^{ns}	60.60**	9.90**	97008**	7041**
a*c	3	274.42 ^{ns}	2.12 ^{ns}	0.72 ^{ns}	266492 ^{ns}	652068**	148 ^{ns}	6.83**	6.89**	61736**	28010**
b*c	6	752.73**	18.73**	4.11**	3702275 ^{ns}	131958**	86.0 ^{ns}	41.02**	9.37**	23087**	11474**
a*b*c	6	795.93**	27.29**	2.78**	3410300 ^{ns}	143614**	69.1 ^{ns}	38.12**	22.66**	29743**	24301**
Y*a	1	1.30**	49.47**	2.20*	455 ^{ns}	4886826**	746**	8.41**	8.99**	582500**	116659**
Y*b	2	374.36 ^{ns}	14.44*	1.69**	455 ^{ns}	214881**	43.8 ^{ns}	0.07 ^{ns}	0.78**	28591**	5268**
Y*c	3	431.92**	7.46 ^{ns}	1.68**	31320 ^{ns}	266211**	46.9 ^{ns}	0.79**	3.08**	30997**	5898**
Y*a*b	2	383.41**	4.43 ^{ns}	0.54 ^{ns}	455 ^{ns}	306261**	50.5 ^{ns}	0.05 ^{ns}	0.42 ^{ns}	40133**	7254**
Y*a*c	3	179.96 ^{ns}	0.40 ^{ns}	0.26 ^{ns}	31320 ^{ns}	326470**	63.9 ^{ns}	0.28 ^{ns}	5.18**	37231**	7045**
Y*b*c	6	143.88 ^{ns}	3.81 ^{ns}	0.47 ^{ns}	31320 ^{ns}	76583**	15.6 ^{ns}	0.49**	2.02**	11303**	2032**
Y*a*b*c	6	95.08 ^{ns}	2.68 ^{ns}	0.40 ^{ns}	31320 ^{ns}	90052**	22.9 ^{ns}	0.65**	2.55**	12678**	2280**
Error	92	177.88	3.98	0.337	2650762	4911	57.0	0.119	0.150	562.567	115
CV%		12.95	11.90	14.67	22.40	13.20	23.80	10.99	12.45	13.20	11.32

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

PH: Plant Height; DM: Days to maturity; SW: 100-Seed Weight; BY: Biological Yield; Y: Grain yield; HI: Harvest Index; PC: Protein Content; OC: Oil Content; PY: Protein Yield; OY: Oil Yield.

Mean comparisons of the traits under applied treatments revealed that humic acid had a significant and positive effect on the measured traits including PH, DM, SW, Y, HI, PC, OC, PY, and OY (Table 4). This treatment led to 65% the increase of seed yield with 2750 kg/Ha, in compare to control. Furthermore, rhizobium increased the soybean traits solely and without azospirillum (Table 4). At this order, the

results showed that rhizobium and rhizobium+azospirillum treatments led to 14 and 9% the increase of seed yield in compare to to control. The foliar application of Co, Mo, and B micronutrients, alone or in combination, increased the soybean traits compared to the control (Table 4). The highest seed yield (2387 kg/h) was observed in the Co+Mo+B combination. The application of Co, Co+Mo and Co+Mo+B

treatments led to 19, 26 and 28% the increase of seed yield in compare to control. The interaction of treatments showed significant effect on seed yield, it was found that under humic treatment, application of bacteria and nutrition can be effective on improvement of seed yield, so the highest seed yield mean was obtained by humic acid + Rhizobium+Azospirillum + Co+Mo+B treatments with 3268 kg/ha value.

In relation to protein and oil content of seeds, it was found that treatments had significant effects on these properties, application of humic acid led to 1.71 and 1.95 fold of protein and oil yield per hectare in compare to control, respectively (Table 5). Also, rhizobium and rhizobium+azospirillum

treatments led to 13 and 4% the increase of protein yield and 23 and 18% the increase of oil yield, in compare to control (Table 5). The highest protein yield (854 kg/h) and oil yield (384 kg/ha) were observed in the Co+Mo+B treatment. According to treatments interaction, it was found that under humic treatment, application of bacteria and nutrients can be effective on improvement of protein and oil yield, so the highest protein yield mean was obtained by humic acid + Rhizobium + Co+Mo+B treatments with 1103 kg/ha value and highest mean of oil yield was obtained by humic acid + Rhizobium+Azospirillum + Co+Mo treatments with 577 kg/ha value (Table 5).

Table 4. The mean comparison of Plant Height, Days to maturity, 100-Seed Weight, Biological Yield, Grain yield and Harvest Index in response to interaction of treatments

			PH (Cm)	DM	SW (gr)	BY (kg/ha)	GY (kg/ha)	HI%
Humic acid	Rhizobium	Co	92.73 bcd	108.3 abc	14.05 ab	5939 a	2948 e	50.31 a
		Co+Mo	109 a-d	107.4 a-d	14.05 ab	6532 a	2967 d	45.54 ab
		Co+Mo+B	119.6 a	108.7 ab	14.82 a	6623 a	3125 b	47.47 ab
		control	94.25 a-d	105.8 b-e	13.53 bcd	8267 a	2406 i	29.32 d-h
	Rhizobium + Azospirillum	Co	119 ab	104.4 cde	13.82 abc	7697 a	2747 g	36.26 a-g
		Co+Mo	117.8 ab	106.8 a-d	13.83 abc	7565 a	2720 h	44.29 abc
		Co+Mo+B	103.3 a-d	110.2 a	13.87 ab	6920 a	3268 a	40.91 a-d
		control	104.6 a-d	102.7 e	11.8 fgh	7007 a	2068 l	30.89 c-h
	Control	Co	90.9 cd	106 b-e	12.74 c-f	7239 a	2741 g	40.03 a-e
		Co+Mo	113.7 abc	105.1 b-e	12.78 c-f	7587 a	2835 f	39.14 a-f
		Co+Mo+B	103.7 a-d	105.6 b-e	11.87 fgh	7683 a	3017 c	40.82 a-d
		control	100.9 a-d	103.7 de	11.26 ghi	6261 a	2162 j	35.76 b-g
Control	Rhizobium	Co	98.23 a-d	105 b-e	12.73 c-f	7200 a	1771 p	24.81 fgh
		Co+Mo	110.9 a-d	104.9 b-e	13.3 bcd	7475 a	1817 o	25.43 fgh
		Co+Mo+B	113.2 abc	105.7 b-e	12.2 efg	7528 a	1929 m	26.8 d-h
		control	111.3 a-d	103.7 de	12.6 def	7580 a	1721 q	23.44 gh
	Rhizobium + Azospirillum	Co	99.4 a-d	105.2 b-e	12.77 c-f	8070 a	1676 r	20.91 h
		Co+Mo	112.6 a-d	105.5 b-e	13.28 b-e	7222 a	1844 n	26.12 e-h
		Co+Mo+B	95.8 a-d	107.3 a-d	13.23 b-e	7642 a	2085 k	28.06 d-h
		control	93.63 a-d	105.4 b-e	9.58 kl	6587 a	1500 s	23.61 gh
	Control	Co	108.2 a-d	104.2 de	10.32 ijk	6418 a	1466 t	23.82 gh
		Co+Mo	107.4 a-d	103.4 de	10.22 jk	7356 a	1386 v	20.29 h
		Co+Mo+B	86.3 de	105.1 b-e	11.13 hij	7643 a	1450 u	20.91 h
		control	65.42 e	95.33 f	8.72 l	8065 a	1288 w	17.14 h

In each column of each treatment, the means with different letters have a significant of 5% in LSD test.

PH: Plant Height; DM: Days to maturity; SW: 100-Seed Weight; BY: Biological Yield; Y: Grain yield; HI: Harvest Index

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Table 5. The mean comparison of PC, OC, PY and OY in response to interaction of treatments

			PC%	OC%	PY (kg/ha)	OY (kg/ha)
Humic acid	Rhizobium	Co	32.45 j	16.18 g	963.1 d	482.8 e
		Co+Mo	35.12 hi	17.92 bcd	1045 b	534.2 b
		Co+Mo+B	35.2 ghi	17 f	1103 a	534 b
		control	36.96 cd	17.76 cde	891.5 e	428.6 g
	Rhizobium + Azospirillum	Co	35.95 efg	18.47 b	989.7 cd	509.1 c
		Co+Mo	30.85 lm	17.65 c-f	1009 bc	577.1 a
		Co+Mo+B	37.15 bcd	17.15 ef	1019 bc	486 de
		control	36.13 ef	17.08 ef	747.6 f	353.8 h
	Control	Co	36.67 de	18.17 bc	1011 bc	502.6 cd
		Co+Mo	38.53 a	15.79 g	1096 a	450.4 f
		Co+Mo+B	36.06 ef	17.39 def	1098 a	539.7 b
		control	34.55 i	13.81 ij	748 f	300.3 ij
Control	Rhizobium	Co	35.73 fgh	19.79 a	633.1 g	350.4 h
		Co+Mo	35.69 fgh	13.83 ij	648.7 g	251.8 lm
		Co+Mo+B	37.63 bc	13.38 j	726.2 f	258.6 kl
		control	35.68 fgh	15.88 g	614.6 gh	274.4 k
	Rhizobium + Azospirillum	Co	31.45 kl	14.19 i	527.3 j	238 m
		Co+Mo	31.69 k	15.96 g	584.6 hi	294.9 j
		Co+Mo+B	35.39 fgh	15.13 h	737.9 f	315.5 i
		control	31.41 kl	13.86 ij	471.4 k	208.2 n
	Control	Co	37.75 b	13.97 ij	553.5 ij	205 no
		Co+Mo	31.61 kl	13.62 ij	438.1 k	188.8 op
		Co+Mo+B	30.5 m	12.03 k	442.2 k	174.7 pq
		control	34.98 hi	12.56 k	450.5 k	161.7 q

In each column of each treatment, the means with different letters have a significant of 5% in LSD test.
 PC: Protein Content; OC: Oil Content; PY: Protein Yield; OY: Oil Yield.

Pearson correlation analysis of the measured traits in soybean revealed the relationship of these traits. The grain yield was significantly correlated with PH, DM,

SW, HI, PC, OC, PY, and OY traits. Furthermore, oil content was significantly correlated with all of the traits except BY (Table 6).

Table 6. Pearson correlation analysis of the soybean traits

Traits	PH	DM	SW	BY	Y	HI	PC	OC	PY	OY
PH	1									
DM	0.53**	1								
SW	0.38**	0.38**	1							
BY	-0.11	-0.16	-0.09	1						
Y	0.39**	0.53**	0.54**	-0.11	1					
HI	0.35*	0.52**	0.51**	-0.37*	0.96**	1				
PC	0.21	0.14	0.08	0.11	0.30*	0.25	1			
OC	0.34*	0.44**	0.50**	0.00	0.70**	0.62**	0.38**	1		
PY	0.39**	0.52**	0.49**	-0.07	0.98**	0.93**	0.48**	0.71**	1	
OY	0.38**	0.53**	0.52**	-0.07	0.98**	0.92**	0.34*	0.82**	0.97**	1

PH: Plant Height; DM: Days to maturity; SW: 100-Seed Weight; BY: Biological Yield; Y: Grain yield; HI: Harvest Index; PC: Protein Content; OC: Oil Content; PY: Protein Yield; OY: Oil Yield.

This study integrated different sources of N and examined the effect of micronutrient foliar applications on soybean agronomic parameters. The N sources including humic acid, bacteria fixing organisms, and also Co, Mo, and B micronutrients had a positive impact on the yield and yield-related traits. Ponte et al. (2019) improved the nutritional quality of soybean seeds by inoculating *Azospirillum brasilense* Az39. Due to these positive effects of shoot inoculation with *A. brasilense* Az39 on the growth and pollination of soybeans, seeds harvested from leafy starch plants had higher nitrogen and protein content than plants inoculated during cultivation. Moreover, Amarilla et al. (2019) investigated the effect of Mo, Co, and B foliar application on soybean properties and claimed that the use of these micronutrients increases soybean yield.

The application of biofertilizers, especially plant growth-promoting bacteria, is the most important strategy in integrated plant nutrition management for a sustainable agricultural system (Sharma, 2003; Barros et al., 2022). Bellaloui et al. (2013) studied the effect of boron foliar application on soybean seed compounds including protein, oil, fatty acids, and sugar compounds under drought stress. They sprayed boron in two stages of flowering and seed filling. The results showed that foliar application of boron increased the boron in leaves and seeds and changed seed composition. Protein, linoleic, and oleic acid were also increased in soybean seeds treated with boron foliar application.

Boron is an important micronutrient whose deficiency causes a severe reduction in crop yield. Boron plays an important role in the metabolism of nucleic acids, carbon hydrates, proteins, indoleacetic acid, cell wall synthesis, membrane flexibility and action, phenol metabolism, lignin biosynthesis, vascular differentiation, and glucose metabolism (Brdar-Jokanović, 2020). This micronutrient is also involved in calcium intake, cell division, flowering, fruiting, carbohydrate and nitrogen metabolism, disease resistance, and aqueous bonds, and acts as a catalyst in many reactions (Marshner, 1995).

Fertilizers are generally used for soybean production, which is contrary to the principles of sustainable agriculture. The high cost of using chemical fertilizers and their adverse effects on the environment highlight the importance of using biological fertilizers. However, soybean plants can meet the need for nitrogen fertilizer if there is a suitable population of major rhizobium bacteria.

CONCLUSIONS

The findings of the present study can open a new perspective on soybean cultivation technology. Because of the significant effects of biofertilization on yield, oil, and protein content in soybean, we recommend the use of these nutrients to promote grain yield and quality. Consequently, the application of nitrogen biofertilizer containing micronutrients in combination with fixing microorganisms is a useful approach to inducing plant growth and development.

ACKNOWLEDGEMENTS

The authors would like to appreciate the officials in the Agricultural and Natural Resources Research and Education Center of Golestan for cooperating in conducting this research project.

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