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Effect of chemical fertilizers and bio-fertilizers application on some morpho-physiological characteristics of forage sorghum

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Abstract

To evaluate effect of chemical and biological fertilizers on growth of the forage sorghum, a factorial experiment was arranged as factorial, based on randomized complete block design with three replications at the research farm of the faculty of agriculture, university of Tabriz, Iran in 2011. Treatments were chemical fertilizers levels (210 Kg/ha urea (100%), 150 Kg/ha triple superphosphate (100%), urea (100%) + triple superphosphate (S.P.T, 100%), urea 50% + S.P.T. 50% and control) and bio-fertilizers (biosuper, phosphate barvar-2, biosuper + phosphate Barvar-2 and control). Results indicated that the highest (3090.99 g.m2) and the lowest (1226/29 g.m2) forage yield and plant height were obtained from "urea (100%) + S.P.T. (100%) + phosphate Barvar-2" and "control", respectively. The highest and lowest of leaf area index (LAI) were achieved in "urea (100%) + S.P.T. (100%) + biosuper + phosphate Barvar-2" and "control "respectively. Chemical and bio-fertilizers had significant effects on Natural Detergent Fiber (NDF), as the control and treatment of "50% urea + 50% S.P.T. + phosphate Barvar-2" produced higher and lower NDF than other treatments respectively. Also the most gas production was observed in "50% urea + 50% S.P.T. + biosuper + phosphate Barvar-2". In conclusion, application of 100% chemical fertilizer with bio-fertilizers resulted the maximum of quantitative values such as forage yield, and the reduced doses of chemical fertilizer (50%) with bio-fertilizers had more positive effects on qualitative traits such as NDF.

Keywords: Biosuper, gas production, LAI, NDF and phosphate barvar-2.

Introduction

Forage sorghum by having the individual physiological characters such as resistance to water deficit and soil salinity, high water use efficiency, high quality of forage and as a silage crop is more interested in arid and semiarid regions (Zerbini and Thomas 2003). Okon (1985), indicated that use of bio-fertilizers instead of chemical fertilizers is not sufficient whereas using of bio-fertilizers increased the efficiency of chemical fertilizers, however, by low using of chemical fertilizers, maximum crop yield achieved. Although the soil is rich in phosphorus but it is not available for crop, though using of phosphor solubilizing bacteria such as *Pseudomonas* bacteria could be an efficient and beneficial practice (Belimov et al., 1995). Rai and Gaur (1988), reported a synergistic effect of *Azospirillum* and *Azotobacter* on the yield of wheat, corn and sorghum. Inoculation by *Azospirillum* increased total dry matter and seed yield in sorghum up to 10-30 percentage compared with control (Kapulnic et al., 1981). Effect of different N fertilizer levels and bio-fertilizers on forage sorghum indicated that using of 75 kg/ha N (urea), 25 kg/ha N (castor residuum) and inoculation by *Azospirillum* increased the raw protein and quality of forage (Yadav et al., 2007). In all sorghum hybrids, about leaf area index, 50-60 days after sowing an increase observed then stabilized within 80 days and then a decrease observed (Bueno and Athins 1982). Van Oosterom et al., (2010), revealed that effect of nitrogen fertilizer on LAI and stem growth of sorghum was significant. It had reported that using of bio-fertilizers such as *Azotobacter* and *Azospirillum* bacteria had a positive effect on sorghum yield (Van Oosterom et al., 2010). In other study, inoculation of seeds by *Azotobacter* and *Azospirillum* increased forage yield of sorghum (Singh et al., 2005). According to Dobbelaere et al., (2002), inoculation of seeds by

Azotobacter increased the yield of cereal up to 30 percentages. Saini et al., (2004), reported that using of 50 percent of chemical fertilizers and farm yard manure with inoculation of seeds by *Rhizobium* bacteria and phosphor solubilizing bacteria, increased grain yield and biomass of sorghum and chick pea. According to Akbari et al., (2009), combination of bio and chemical fertilizers increased grain yield, plant height, biological yield and harvest index of sunflower. In this sense, this study was undertaken to evaluate the effects of application of bio and chemical fertilizers and introducing of suitable composition or combination treatments to achieve high performance in sorghum.

Material and methods

The field study was conducted at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran (38°5N, 46°E) in 2011. Experiment was arranged as factorial, based on randomized complete block design in three replications. Treatments were chemical fertilizers (210 Kg/ha urea (100%) (a1), 150 Kg/ha triple superphosphate (100%) (a2), urea (100%) + triple superphosphate (S.P.T, 100%) (a3), urea 50% + S.P.T. 50% (a4) and control (a5), and bio-fertilizer (biosuper (b1), phosphate barvar-2 (b2), biosuper + phosphate barvar-2 (b3) and control (b4)). Chemical fertilizers applied based on soil analysis results (Table 1). Nitrogen fertilizers were used in three equivalent parts (before sowing time, after first harvest and after second harvest) and triple superphosphate was completely used before sowing time. Each plot consists 4 rows of 4 m long with inter-row (50cm) and intera-row (10 cm) distances that established 20 plant/m^2 populations. Seeds of *Sorghum bicolor cv.* Speed feed were sown at 2-3 cm depth. Optimum density for forage sorghum was 20 plant/m^2. Biosuper fertilizer consists of *Azotobacter*, *Azospirillum, Pesudomonas* and *Bacillus* Bacteria, and phosphate barvar-2 consists of *Pesudomonas* and *Bacillus* Bacteria. Weeds controls were regularly performed by hand and irrigation was performed once every 7 days. Natural detergent fiber measured according to Van Soest and Wine (1967) methods. Fedorak and Hrudey (1983) water displacement technique were used for gas production measurement. In this method, water displacement in a test tube with glasses containing rumen fluid and food samples, indicate the rate of gas production. Foods were ground evenly by mill having sieve pores with a diameter of 2 mm then 300 mg of each grounded foods weighted carefully and transferred into a sterile 50 ml glass serum. For each food sample three replications were considered. Rumen fluids required in the test gas production were gathered from two fistula sheep two hours after the morning meal feed. The rumen fluid before moving into glass serum, were mixed buffer prepared by Mcdougall method (1948) at 1 to 2 ratios (a section of the rumen fluid and 2 section of the buffer). 20 ml mixture of rumen fluid and buffer were added each test tube and transferred into the shaker incubator at 120 rpm and at 39 ° C. The amount of gas produced from the fermentation of food was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h after incubation, respectively.

Evaluating factors were forage yield, plant height, leaf area index, gas production, and natural detergent fiber. Analysis of variance and comparison of means were performed using MSTAT-C software. Excel software was used to draw figures.

Results and discussion

Dry forage yield

Analysis of variance of the data (Table 2) showed, the main effects of chemical fertilizers and bio-fertilizers and interaction of chemical and bio-fertilizers were significant ($P \leq 0.01$) Means comparisons revealed that the highest yield (3901 g/m^2) was obtained from "urea+ triple superphosphate+ phosphate Barvar 2" treatment, and the lowest yield (1226 g/m^2) belonged to control treatment. Application of biosuper and phosphate Barvar 2 in compare of control increased forage yield (108 and 71% respectively). Although seeds inoculation by *Azospirillum* and *Bacillus* bacteria in the presence and absence of nitrogen and phosphate fertilizers increased dry yield weight and grain yield, however, at inoculated treatments, nitrogen and phosphate uptake had increased (Alagawadi, and Gaur 1992). Effect of different chemical fertilizers, cattle manure, *Azospirillum* Bacteria and phosphor solubilizing bacteria on rainfed sorghum was meaningful. Similary Ponnuswamy et al., 2002 noted that, the highest performance of forage and grain yield was achieved in incorporation of 100% NPK cattle manure (10 t.ha^{-1}) Azospirillum Bacteria and phosphor solubilizing bacteria. These results were also agreed with Alagawadi and Gaur (1992), studies on forage sorghum.

Plant height

Analysis of variance for plant height (Table 2), revealed that effects of chemical fertilizers and interaction of bio and chemical fertilizers were statically meaningful (1%, 5% respectively). Results showed that maximum and minimum plant height was observed in "urea+ triple superphosphate + phosphate Barvar 2" and control treatments (133 and 94.11 cm respectively). According to table 4, application of bio-fertilizers such as biosuper and phosphate Barvar 2 compared with control treatment increased the plant height (18.41%, 15.10% respectively). Plant dry weight, height, leaf area index, leaf area ratio and specific leaf weight of grain sorghum were increased by using of bio-fertilizers such as *Azotobacter, Pesudomonas* Bacteria. Alnoaim and Hamad (2004), reported that by using of bio-fertilizers with using of N fertilizer (180 kg.ha-1) the highest plant height, number of tiller and grain yield of rice (*Oryza sativa*) were achieved.

Cell wall

Analysis of variance of data (Table2), showed that main effects of chemical fertilizers and bio-fertilizers and interaction of chemical and bio-fertilizers were significant ($P \leq 0.01$) According to the figure 3, comparison of data revealed that the highest cell wall percentage (62.85%) were obtained from "urea 50% + S.P.T. 50% + phosphate Barvar 2" and the lowest cell wall percentage (55.9%) belonged to control treatment. Using of urea fertilizer in 200 $kg.ha^{-1}$ ratio decreased the fiber mounts of sorghum. In other hand, by increasing of N levels, raw fiber value reduced and subsequently suavely and digestibility of forage increased (Almodares et al., 2009). Application of *Pesudomonas* bacteria decreased the soluble natural detergent fiber (NDF) up to 50.4 %, however, the lowest soluble natural detergent fiber were achieved by using of triple superphosphate in 60 $kg.ha^{-1}$ ratio (Mehrvarz and chaichi 2009).

Gas production rate

Gas production was calculated by sum of the gas production hours, thereby previous produced gas amounts were added to later hour's products. In this study the effective hours which had maximum and minimum gas production was compared. According to the table 4, interaction of chemical fertilizers and bio-fertilizers about gas production rate was statically meaningful ($P \leq 0.01$) With respect to the means of data, at all hours of gas production, the highest and lowest gas were produced from urea 50% + S.P.T. 50% + biosuper + phosphate Barvar 2 (231.4 mg dry matter) and triple superphosphate + biosuper + phosphate Barvar 2 (85.48 mg dry matter) treatments respectively (Figure 1). Results showed, using of bio-fertilizers with half recommended rate of chemical fertilizers could promote forage suavely and digestibility which is the perfect step in development of sustainable agriculture and important practice in minimizing of environmental pollution. High gas production rates reflect the high energy of metabolism, zymotic nitrogen and other nutrient elements which are necessary for microorganism's activity (Datt and Singh 1995). During the anaerobic digestion process in rumen of animals, volatile fatty acids, CO_2, methane and a low mounts of hydrogen are produced. Measurement of gas production is the best practice for determination of suavely and digestibility of forage (Menke et al., 1979).

Leaf area index (LAI)

Leaves are usually the main resources of plant photosynthesis. Wilcox (1985), reported that leaf area index is the best indicator for dry matter production. In general after germination phase, LAI by a soft and slow rate is increase then with warming of weather the rapid expansion of leaves phase commence. By increasing of LAI, light reception and dry matter production due to the canopy formation is increase. Due to the decreasing in light infiltration to the bottom of canopy, photosynthesis activity is decrease. To determine a mathematical model express leaf area index changes over the next days after sowing, Excel software were used for determination of polynomial equations and the best equations was : $LAI = \exp(a + bx + cx^2)$
LAI: Leaf area index, x: Days after sowing and a,b,c: Equation coefficients

Results showed that the relationship between leaf area index of sorghum and days after sowing is quadratic equation (Table 5). The maximum LAI (3.48) were observed from "urea+ triple superphosphate + phosphate Barvar2+ biosuper" and minimum LAI (0.95) belonged to control treatment (Figures 2-6). Treatment of biosuper and phosphate Barvar 2 during the growth period compared with control had the highest LAI. Also low LAI was observed in treatment of urea 50% + S.P.T. 50% + phosphate Barvar 2 in the early stages of growth, however, by affecting of bio-fertilizers leaf area index increased. Using of different levels of fertilizers had significant effect on leaf area index of corn (Rasheeed et al., 2003). According to Colomb et al., (2000), by increasing of phosphor levels, plant growth, LAI, photosynthesis and yield of corn (*Zea mays*) increased. Yadav et al (2002), revealed that application of incorporation chemical and bio-fertilizers increased the biological yield, grain yield and LAI of blond plantago (*Plantago ovate*). In other study, application of incorporation chemical and bio-fertilizers on corn crop increased plant height, LAI, dry matter production, leaf area duration (LAD) and leaf area ratio compared with sole chemical fertilizers (Eidi zadeh et al., 2010).

Conclusion

The results showed that combined use of bio-fertilizers with chemical fertilizers increased the evaluated characters. Hence the use of biological fertilizers also significantly reduce the consumption of chemical fertilizers and reduce the adverse environmental effects, however, a good performance can be achieved in forage sorghum plant.

Table 1. Physical and chemical properties of experimental soil before planting

Texture	EC (µs/cm)	pH	Clay%	Silt %	Sand%	OC%	N%	P (ppm)	K (ppm)
Loam-sand	512	7.82	16	22	62	0.74	0.08	61	304

Table 2. Variance analysis of bio and chemical fertilizers effect on qualitative and quantitative characters of forage sorghum

S.O.V$^{\alpha}$	df	Mean squares (MS)		df	MS
		Forage yield	plant height		NDF
Replication	2	1060790.707**	4.663^{ns}	1	1.521*
Chemical fertilizer(A)	4	1956411.091**	612.427**	4	7**
Biological fertilizer(B)	3	329094.809**	14.566^{ns}	3	5.397**
A×B	12	1089278.116**	136.879*	12	15.867**
Error	38	12275.487	63.187	19	0.317
Coefficient of Variation (%)		4.63	6.86		0.95

**, * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

Table 3. Means of bio and chemical fertilizers effect on qualitative and quantitative characters of forage sorghum

Fertilizer	bio fertilizer	Forage yield (g.m2)	plant height (cm)	NDF (%)
Urea	Biosuper	2081.77 h	114 bcd	58.7 d
	Barvar-2	2056.48 h	120 abcd	56 gh
	Biosuper+Barvar-2	2740.61 cde	108.85 cd	62.75 a
	Control	2905.48 bc	119.37abcd	56.75 fgh
Triple superphosphate (S.P.T)	Biosuper	1850.69 ij	114. 07bcd	60.95 bc
	Barvar-2	1699.43 j	105.33 e	۵۶.65 fgh
	Biosuper+Barvar-2	2636.35 def	115.81 bcd	59.9 c
	Control	3033.22 b	123.81 abc	57.15 fgh
Urea+(S.P.T)	Biosuper	2756.16 cd	122.59 abc	57.2 efgh
	Barvar-2	3900.65 a	133.29 a	60.95 bc
	Biosuper+Barvar-2	3090.99 b	127.03 ab	62.8 a
	Control	2352.35 g	118.77 abcd	58.4 de
50% Urea + 50% (S.P.T)	Biosuper	1806.53 ij	115.63 bcd	57.95 def
	Barvar-2	1986.36 hi	119.51 abcd	55.9 h
	Biosuper+Barvar-2	2461.17 fg	117.14 bcd	57.1 efgh
	Control	2836.72 c	119.51 abcd	62.6 a
Control	Biosuper	2551.19 ef	111.44 bcd	60 c
	Barvar-2	2097.69 h	108.33 cde	61.9 ab
	Biosuper+Barvar-2	1781.36 j	108.18 cde	57.3 efg
	Control	1226.29 k	94.11 e	62.85 a

Means within a column followed by the same letters are not significantly different at α=0.05

Table 4. Variance analysis of the chemical fertilizers and bio fertilizers on gas production

		Mean squares (MS)										
		Gas production after incubation (mg dry matter)										
S.O.V$^{\alpha}$	df	2	4	6	8	12	16	24	36	48	72	96
Replication	2	0.096^{ns}	5.685^{ns}	14.645^{ns}	2.151^{ns}	11.096^{ns}	24.27^{ns}	17.062^{ns}	61.418^{ns}	6056.683^{ns}	743.311^{ns}	1130.651^{ns}
Chemical fertilizer(A)	4	24.61^{ns}	51.626^{ns}	117.79^{ns}	52.058^{ns}	113.783^{ns}	245.091^{ns}	946.279^{ns}	2702.45^{ns}	3556.628^{ns}	2168.02^{ns}	1842.086^{ns}
Biological fertilizer(B)	3	24.49^{ns}	36.726^{ns}	639.635**	88.245^{ns}	84.157^{ns}	184.894^{ns}	1212^{ns}	2731.024^{ns}	3116.099^{ns}	3844.605^{ns}	4850.604^{ns}
A×B	12	65.141**	190.474**	175.349^{ns}	170.808^{ns}	426.071**	1042.368**	3867.384**	5780.97**	7128.451**	6800.081**	7167.012**
Error	38	9.45	26.68	101.504	104.838	126.094	273.323	613.538	1055.45	1570.861	2075.356	2021.349
CV (%)		51.10	58.46	36.03	24.6	23.48	28.95	32.81	31.85	29.56	28.84	26.75

**, * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

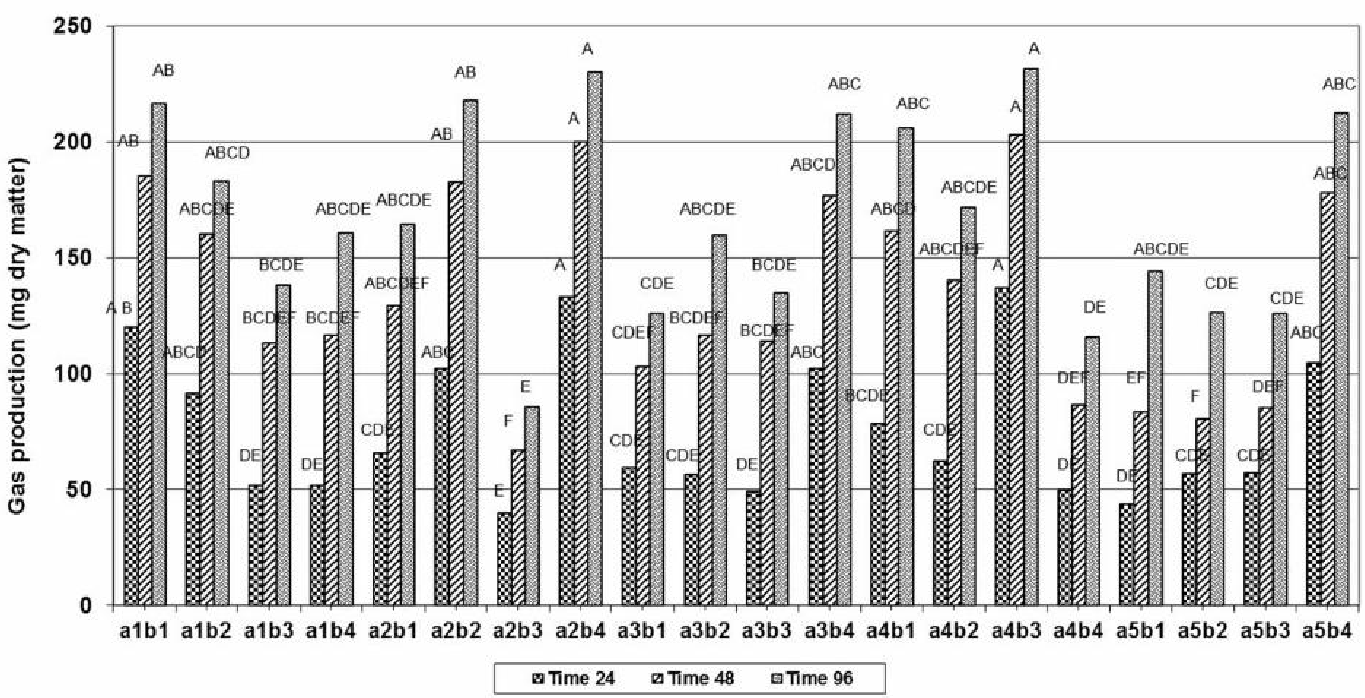

Figure 1. Comparison of the effects of chemical fertilizers and bio-fertilizers on gas production over 24, 48 and 96 h after incubation

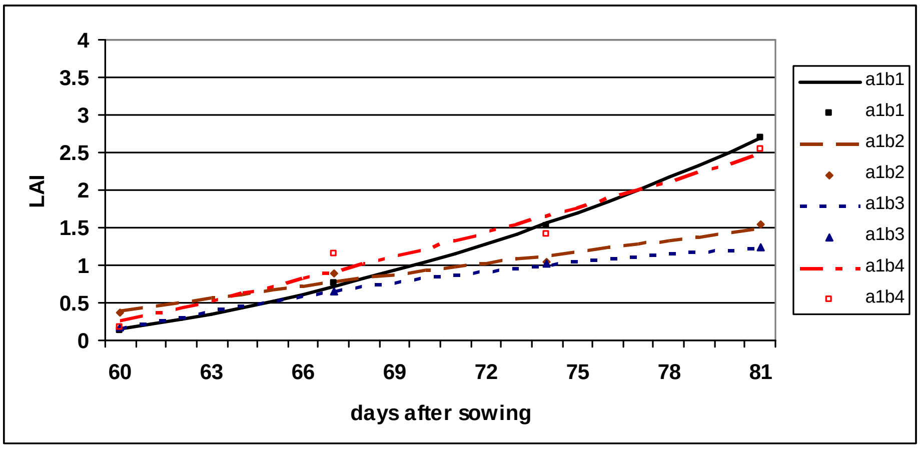

Figure 2. Changes in leaf area index of sorghum treated by urea fertilizer at different levels of bio-fertilizers. a1: urea100%, b1: biosuper, b2: phosphate Barvar 2, b3: biosuper+ phosphate Barvar 2 and b4: control

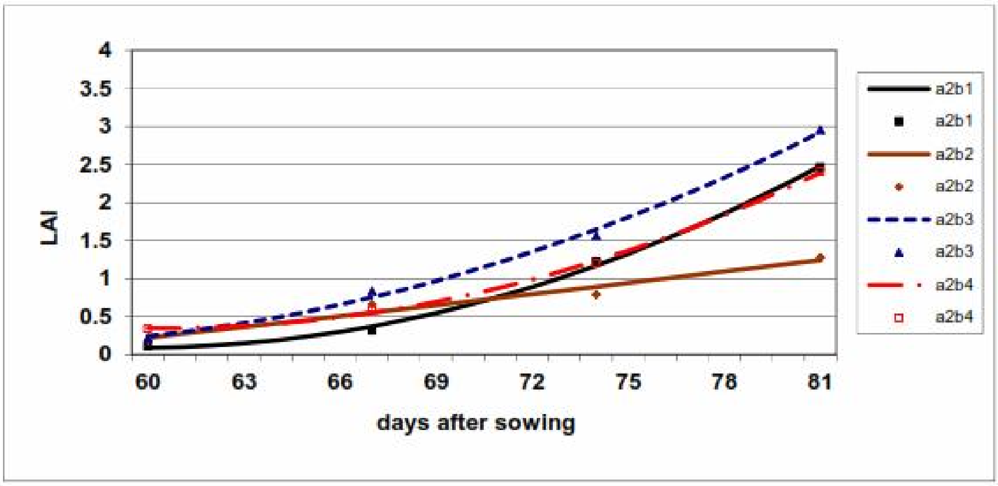

Figure 3. Changes in leaf area index of sorghum treated by triple superphosphate fertilizer at different levels of bio-fertilizers.a2: triple superphosphate 100%, b1: biosuper, b2: phosphate Barvar 2, b3: biosuper+ phosphate Barvar 2 and b4: control

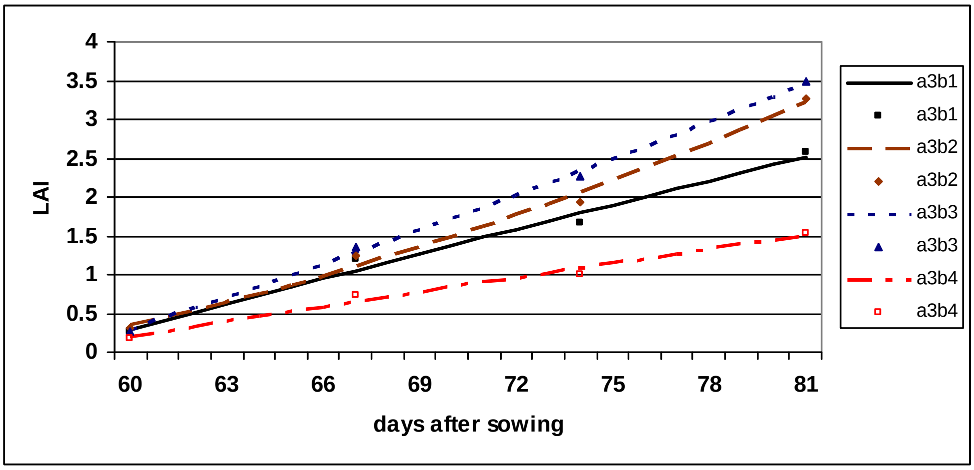

Figure 4. Changes in leaf area index of sorghum treated by urea + triple superphosphate fertilizer at different levels of bio-fertilizers. a3: urea 100% +super phosphate 2 100%, b1: biosuper, b2: phosphate Barvar 2, b3: biosuper+ phosphate Barvar 2 and b4: control

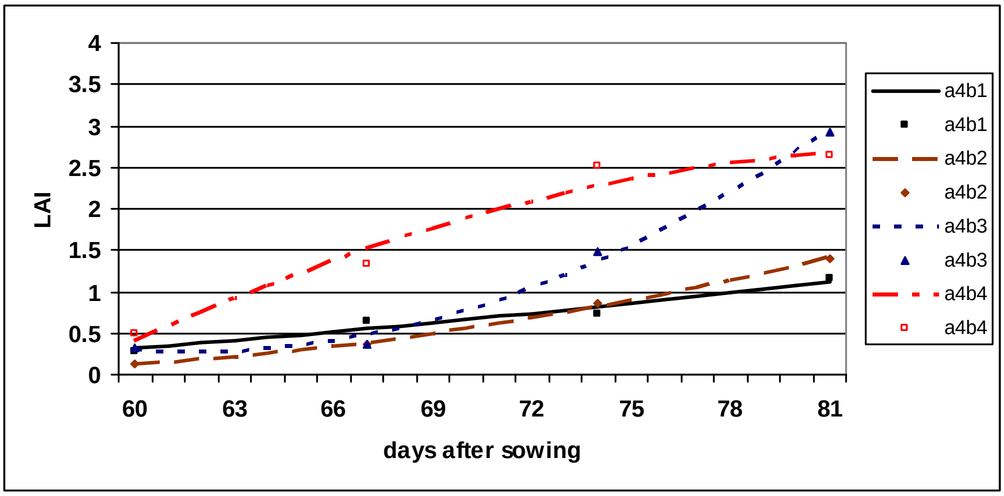

Figure 5. Changes in leaf area index of sorghum treated by 50% urea + 50% of triple super phosphate fertilizer at different levels of bio-fertilizers. a4: urea50%+50%super phosphate 2, b1: biosuper, b2: phosphate Barvar 2, b3: biosuper+ phosphate Barvar 2 and b4: control

Table 5. Coefficients of polynomial equation LAI changes to days after sowing of sorghum at different treatments

treat	intercept (a)	liner regression coefficient (b)	Quadratic regression coefficient (c)	Coefficient of determination (r^2)
a1b1	6.955806	-0.28689	0.002891	0.99
a1b2	-3.71831	0.081096	-0.00021	0.96
a1b3	-9.71031	0.249106	-0.00141	0.99
a1b4	-2.12932	-0.00902	0.000813	0.95
a2b1	18.84491	-0.62841	0.005264	0.99
a2b2	-2.23463	0.035219	0.0000952	0.95
a2b3	11.62794	-0.42483	0.003918	0.99
a2b4	18.31477	-0.59332	0.004898	0.99
a3b1	-6.88854	0.129919	-0.00017	0.98
a3b2	1.702669	-0.14045	0.001964	0.99
a3b3	-5.31299	0.051426	0.000702	0.99
a3b4	-3.96226	0.74736	-0.000088	0.98
a4b1	-0.15923	-0.0146	0.000376	0.94
a4b2	4.770965	-0.18003	0.001711	0.99
a4b3	27.22187	-0.87551	0.007111	0.99
a4b4	-23.2982	0.60755	-0.00354	0.96
a5b1	0.278671	-0.0321	0.000524	0.99
a5b2	-20.9792	0.575704	-0.0037	0.96
a5b3	-5.28422	0.12652	-0.00056	0.96
a5b4	3.824527	-0.13475	0.001228	0.99

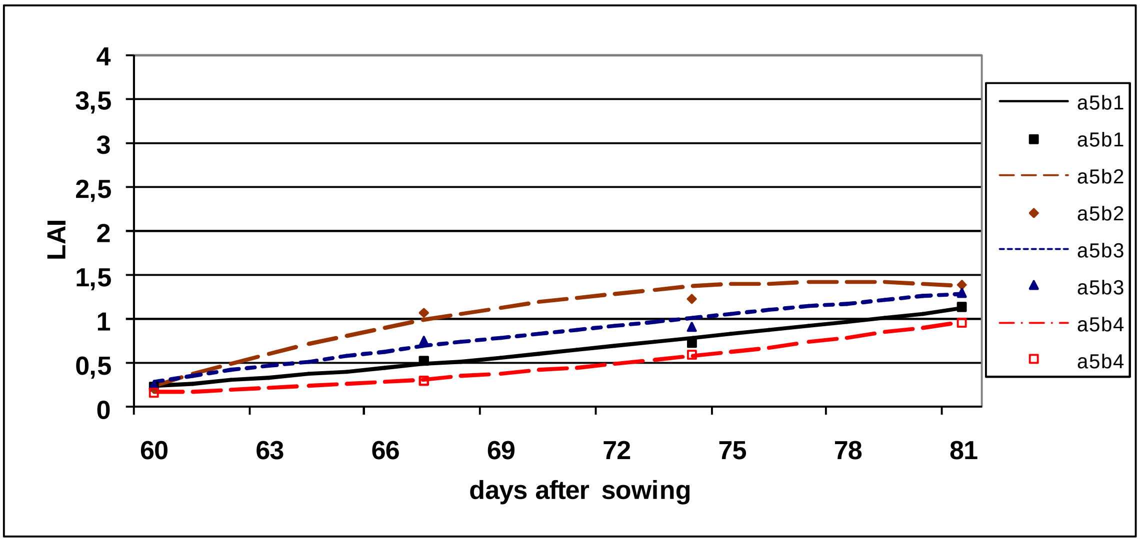

Figure 6. Changes in leaf area index of sorghum at different levels of bio-fertilizers. a5: control, b1: biosuper, b2: phosphate Barvar 2, b3: biosuper+ phosphate Barvar 2 and b4: control

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