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Effect of nano and biological fertilizers on carbohydrate and chlorophyll content of forage sorghum (*Speedfeed hybrid*)

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Abstract

Integrated nutrient management strategies involving chemical and biologic fertilizer is a real challenge to stop using the high rates of agrochemicals and to enhance sustainability of crop production. A factorial greenhouse experiment was arranged based on completely block design in three replications. First factor included in two level: manure fertilizer and none manure fertilizers application and second factor in four chemical fertilizers levels : control (no application of chelate nano-fertilizers),iron chelate nano-fertilizer , potassium chelate nano-fertilizer and combined iron and potassium chelate–fertilizers and third factor in four levels: control (no biofertilizer application), Azetobarvar 1 (*Azetobacter* in 108-107CFU/gr), Phosphatebarvar 2 (*Pseudomonas putida*, Strain P13+ *Pantoea agglomerans*, Strain P5) and combined Azetobarvar 1 and Phosphatebarvar 2. Results showed that manure and nano fertiizers had significant effect on chlorophyll a, b, carotenoid ($p \leq 0.01$). Biofertilizer also affect Chlorophyll b, carotenoid ($p \leq 0.01$) and carbohydrate ($p \leq 0.05$). According to results, the highest chlorophyll a (1.59 mg/g), chlorophyll b (5.31 mg/g), carotenoid (2.24 mg/g) and carbohydrate (3.24 mg/g) were achieved from combine biofertilizers (azetobarvar 1+ phosphorbarvar 2) + chelated nano fertilizers (Fe+k) treatments application. This study showed that these types of fertilizers could promote studied values in sorghum plant.

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Introduction

Sorghum (*Sorghum bicolor* L.) is a warm-season (C4 photosynthetic pathway), short-day annual grass. It grows best under relatively high temperatures and under sunny conditions. Sorghum as a crop originated as far back as 3,000 years ago. The selection in those early times was for grain more than for forage. However, selection for forage varieties has been occurring for the last hundred years. Forage sorghums are similar to grain types but are taller and have higher forage quality (Dahlberg, 2000).

Nowadays, world's attention (permanent agriculture) to stable agriculture and applying new technology and science in farms to minimize damage to resources and maximum utilization of it, is considered. (Gholami *et al.*, 2013). Use of inorganic fertilizers has increased considerably to meet the higher nutrient requirements of the present day improved varieties. However, ecological and environment concerns over the increased and indiscriminate use of inorganic fertilizers have made research on use of organic materials as a source of nutrients very necessary (Upadhyaya *et al.*, 2003). A number of diverse organic sources are available for use in agriculture. Organic manures like farm yard manure (FYM), poultry manure (PM) and vermicompost (VC) can play important role productivity. These sources can reduce the mining of soil nutrient and improve soil organic matter, humus and overall soil productivity (Kumar *et al.*, 2013).

The application of fertilizers is one of the primary methods for improving the availability of soil nutrients to plants. Fertilizing can change rates of plant growth, maturity time, size of plant parts and phytochemical content of plants (Mevi-Schütz *et al.*, 2003). High-input practices such as heavy use of chemical fertilizers have created a variety of economic, environmental, ecological and social problems. Furthermore, the increasing costs of chemical inputs have left farmers helpless, resulting to decreasing seed quality of certain crops and resulting in the fall of commodity prices and consequently reducing farm income (Khadem *et al.*, 2010). In such situation the organic manure play a major role in order to achieve sustainable agriculture. Organic manure is a suitable source of macro- and micronutrient; additionally, using it as fertilizer can be an important disposal method (Taheri *et al.*, 2011). Manure has always been considered as a valuable input to the soil for crop production. In a broad sense, manure management relates to the appropriate use of animal manure according to each farm's capabilities and goals while enhancing soil quality, crop nutrition, and farm profits (Karmakar *et al.*, 2007). Organic fertilizers is a very important method of providing plant with their nutritional requirements without having the best undesirable impact on the environment. Cattle manure is the source of N and other nutrients for plants (such as phosphorus, potassium, calcium, iron, zinc and copper) that can make valuable contributions to soil's organic matter, can improve physical fertility, and is a center for biological activities (Najm *et al.*, 2012). Organic manure and biofertilizers are important for crops to product best product in both quantity and quality and it is also safe for human, animal and the environment (Subba rao, 1993). Applying of organic manures and biofertilizers such as cattle manure and nitrogen fixing bacteria has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety (Mahfouz and Sharaf Eldin, 2007).

The term biofertilizer or called 'microbial inoculants' can be generally defined as a preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms used for application of seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (Mohammadi and Sohrabi, 2012). Azotobacter is used as biofertilizer in the cultivation of most crops (Yasari *et al.*, 2007). It can successfully grow in the rhizospheric zone of wheat, corn, rice and many other crops (Jadhav *et al.*, 1987). There is numerous papers that reported the positive effect of biofertilizers on crop values for example Amal *et al.*, (2010) reported that there were significant positive differences of growth characters and yield and its components except specific leaf area and harvest index obtained when plant were applied by bio-fertilizer. In other study 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 (Eshaghi sardrood *et al.*, 2013). About chlorophyll content, applying biological fertilizers specially vermicompost has better impact that chemical fertilizers and Vermicompost+ N80P80 had better impact on chlorophyll content in comparison with other experimental treatments (Mousavi Nik *et al.*, 2011). Rahi (2013) reported that increase in nitroxin also increased fresh and dry weights of leaf, stem, chlorophylls a, b, total carotenoids, and anthocyanin content of the plants linearly. The highest values of vegetative growth, oil yield, chlorophyll content and NPK percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer (Ahmed *et al.*, 2013). Total chlorophyll content in leaf was increased by inoculation of biofertilizers. Inoculation of *Pseudomonas striata* (PSB)+VAM fungi+*Azotobacter* was recorded significantly higher chlorophyll content in leaf, as compared to PSB+*Azotobacter* and VAM+ *Azotobacter* inoculation (Patra *et al.*, 2013).

Nano-fertilizers are designed in order to gradually release their food contents. They are also manufactured in such a way that the time of their liberation matches with the food product requirement. The use of nano-fertilizers leads to an increase in the efficiency of the consumption of food elements (Rezaei and Abbasi, 2014). Nanotechnology as a new powerful technology has the ability to create a great revolution and transformation in food supply system in a global scope (Sharma *et al.*, 2005). Iron chelate Nano fertilizer can be considered as a rich and reliable source of bivalent iron for plant because of its high stability and gradual release of iron in a wide pH range (3 to 11). One advantage of this nano fertilizer is using no ethylene compounds in its structure. Ethylene enhances growth process and prevents appearing indications caused by chlorosis in leaves (Monsef-Afshar *et al.*, 2013). Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants (Nadi *et al.*, 2013). Moreover, the most evident effect of iron deficiency is a decreased content of photosynthetic pigments, which results in the relative enrichment of carotenoids over chlorophylls and leads to the yellow color that is characteristic of chlorotic leaves (Pirzad, 2010). Some researchers studied in this field; for example positive effect of different iron concentration treatments on leaf chlorophyll was reported by Nadi *et al.*, (2013) and explained that Spraying 6 (g/L) concentration of nano iron had the highest (45.2 SPAD) chlorophyll content. According to Shaviv *et al.*, (1997) nano-sizing, makes fertilizer nutrients more available to nano scale plant pores, and therefore result in greater nutrient use efficiency. Different iron concentration treatments on leaf chlorophyll were significant (Rezaei and Abbasi, 2014). Yield difference between nano-K and muriate of potash (MOP) treated plots was significant and the yield increase by nano-fertilizer over MOP at 20 kg K_2O/ha is 8 %.

Despite of extensive
researches about the effects of organic matter, plants growth promoting microorganisms and nano fertilizers on quantitative and qualitative characteristics of different plants; the combined effect of these fertilizers on characteristic of forage sorghum have not been considered by researchers. Then it need be understood.

The aim of this study was evaluation the effect of nano and biological fertilizers on carbohydrate and chlorophyll content of forage sorghum (*Speedfeed hybrid*).

Material and methods

Seed sowing and treatments

Pots in 4 kg sizes were filed by sandy loam soil and seeds were sown in 2-3 cm deep and irrigated regularly. Regard to recommend of Khazra company iron and potassium chelate nano-fertilizers were used as 0.002375 and 0.00475 g/kg respectively. Livestock manure and nano fertilizers were mixed by soil before the sowing and biofertilizers regard to recommend of Zist Fannavar Sabz company were applied by irrigation water after seeds germination.

Measurement of chlorophyll and carbohydrate

Evaluated values in this study were chlorophyll content (a, b and carotenoids) and carbohydrate. Arnon (1994), Lichtenthaler and Wellburn (1983) and Paquin and Lechasseur, (1979) methods were used for measuring of Chlorophyll a, b, carotenoids and carbohydrate respectively.

Statistical analysis

A factorial greenhouse experiment was arranged based on completely block design in three replications. First factor included in two level: manure fertilizer and none manure fertilizers application and second factor in four chemical fertilizers levels : control (no application of chelated nano-fertilizers), iron chelate fertilizer , potassium chelate nano-fertilizer and combined iron and potassium chelate nano-fertilizers and third factor in four levels: control (no biofertilizer application), Azetobarvar 1 (*Azetobacter* in 108-107CFU/gr), Phosphatebarvar 2 (*Pseudomonas putida*, Strain P13+ *Pantoea agglomerans*, Strain P5) and combined Azetobarvar 1 and Phosphatebarvar 2. The data were analyzed by MSTAT-C and SAS 9.1 softwares and the figures were drawn by Excel 2010.

Results and discussion

Analysis of variances

Analysis of variances of chlorophyll a, b, carotenoid and carbohydrate (table 1), showed that manure and nano fertilizers had significant effect on chlorophyll a, b, carotenoid ($p \leq 0.01$). Biofertilizer also affect Chlorophyll b, carotenoid ($p \leq 0.01$) and carbohydrate ($p \leq 0.05$). Simple effect of nano fertilizers and biofertilizers on carbohydrate was significant at $p \leq 0.05$ and $p \leq 0.01$ respectively. Moreover, interaction of manure×biofertilizers and nano fertilizers× biofertilizers were effective on studied values than manure×nano fertilizers.

Table 1. Analysis of variances of chlorophyll a, b, carotenoid and carbohydrate.

S.V	df	Chlorophyll a	Chlorophyll b	carotenoid	carbohydrate
Manure (a)	1	1.23**	6.42**	1.61**	0.1
Nano fertilizers (b)	3	1.08**	9.71**	1.23**	0.51*
Biofertilizers (c)	3	0.16	1.5*	0.67*	0.93**
a×b	3	0.71	2.6	0.16	0.42*
a×c	3	0.44**	2.1*	0.61*	0.31
b×c	9	0.33**	2.42**	0.46**	0.34*
a×b×c	9	0.38	1.72	0.29	0.31
error	-	0.19	0.66	0.21	0.39
CV (%)	-	17.52	18.73	16.22	16.87

** , * Significant at $p \leq 0.01$ and $p \leq 0.05$.

Interaction of manure and biofertilizer

According to means comparison of interaction of manure and biofertilizer on chlorophyll a, b, carotenoid and carbohydrate (table 2), the highest chlorophyll a (1.39 mg/g), chlorophyll b (4.49 mg/g), carotenoid (1.79 mg/g) and carbohydrate (3.07 mg/g) were achieved from combined azetobarvar1 and phosphatbarvar2 biofertilizers without manure treatments application.

Rajendran *et al.*, (2008) reported that using of biofertilizers such as rhizobium and *bacillus* could increase leaf chlorophyll content. In other study Lima (1999) indicated that phosphorous deficit will decrease leaf chlorophyll and fluorescence which it could be recover by biofertilizers and manure. According to our results it could be suggested that using of microorganisms could be useful if the environment condition be optimum. These microorganisms need to organic matter for their life which in this condition organic matter could supply their needs (Javaid and Shah, 2010). Presence of organic matter increase microorganism population and accelerate decomposing process (Yan and Xu, 2002). This process release more minerals for plant which it cause to better plant growth and yield (Daly and Stewart, 1999). But in this experiment, treatments of manure reduced beneficial effects of microorganisms on carbohydrate and chlorophyll content of forage sorghum. It seems that presence of manure in bed culture have antagonistic effects on beneficial activity of *Azetobacter*, *Pseudomonas putida* and *Pantoea agglomerans* microorganisms.

Table 2. Means comparison of interaction manure and biofertilizer on chlorophyll a, b, carotenoid and carbohydrate.

Manure treatment	Biofertilizer*	Chlorophyll a (mg/g)	Chlorophyll b(mg/g)	Carotenoid (mg/g)	Carbohydrate (mg/g)
Non manure treatment	control	1.00e	3.88c	1.20d	2.83d
	N	1.21c	4.11b	1.22c	2.89c
	P	1.23b	4.17b	1.56c	2.94b
	N +P	1.39 a	4.49a	1.79a	3.07a
Manure treatment	control	0.7 h	2.20g	1.05g	2.16g
	N	0.93g	3.61f	1.13f	2.62f
	P	0.94ef	3.64e	1.17e	2.75e
	N +P	1.05d	3.81d	1.22c	2.77e

*N, azetobarvar 1; P, phosphorbarvar 2
Different letters in each column indicate significant difference at $p \leq 0.05$.

Interaction of biofertilizer and chelated nano fertilizer

Table 3, shows means comparison of interaction biofertilizer × chelated nano-fertilizer on chlorophyll a, b, carotenoid and carbohydrate. According to results, the highest chlorophyll a (1.59 mg/g), chlorophyll b (5.31 mg/g), carotenoid (2.24 mg/g) and carbohydrate (3.24 mg/g) were achieved from combined biofertilizer (azetobarvar 1+ phosphorbarvar 2) + chelated nano-fertilizers (Fe+k) treatments application. Regard to two comparison tables (Table 2 & 3) it seems that biofertilizers+nano fertilizers had more effect on chlorophyll content and carbohydrate rate compare to manure+biofertilizers treatments. Availability of nutrient minerals such as N, Fe, K, P by both application of bio and nano fertilizers caused to increase in chlorophyll content. Positive correlation of nitrogen and chlorophyll is previously reported by some researchers (Ding *et al.*, 2005; DaMatta *et al.*, 2002). De Rosa *et al.*, (2010) also revealed that combined application of biofertilizers with other fertilizers could improve physiological characteristics of crops. This is agree with our results that integrated nutrient managements could supply forage sorghum nutrient needs and make it independ to external inputs which has negative effects on environment; this fact is the main goal of sustainable agriculture.

Table 3. Means comparison of interaction biofertilizer and chelated nano fertilizer on chlorophyll a, b, carotenoid and carbohydrate.

Biofertilizer	chelated nano fertilizer	Chlorophyll a (mg/g)	Chlorophyll b(mg/g)	Carotenoid (mg/g)	Carbohydrate (mg/g)
control	Control	0.76 l	1.32n	0.69m	2.25 l
	Fe	0.98h	2.22m	1.00 l	2.53h
	K	1.00e	2.00m	1.20g	2.57h
	Fe+K	1.2ef	2.01m	1.22fg	2.64g
Azetobarvar1	Control	0.95h	2.03 l	1.16gh	3.00d
	Fe	1.24d	3.29gh	1.23f	3.03c
	K	1.2e	3.51g	1.22f	3.01c
	Fe+K	1.32c	4.2d	2.06c	3.02c
Phosphorbarvar2	Control	0.98h	3.12h	1.15g	2.71f
	Fe	1.12e	3.22h	1.20f	2.79e
	K	1.16e	3.81f	1.22f	2.92d
	Fe+K	1.30c	4.49c	1.61d	3.01c
Azetobarvar1 Phosphorbarvar2	Control	1.1g	4.00e	1.34e	3.02c
	Fe	1.46b	4.75c	1.36e	3.14b
	K	1.51b	5.00b	2.16b	3.15b
	Fe+K	1.59a	5.31a	2.24a	3.24a

Fe, iron chelate nano fertilizer; K, potassium chelate nano fertilizer
Different letters in each column indicate significant difference at $p \leq 0.05$.

Conclusion

It could be concluded that in arid and semiarid area of Iran, these types of biofertilizers without any contamination of environment by using synergic effect of fertilizers, could supply plant required nutrition and by maintain of soil microorganism the better condition for plant growth is prepare. In this study effect of nano fertilizers on chlorophyll content was studied while there is a few papers that investigate such fertilizers on chlorophyll content and carbohydrate rate. This study showed the potential of integrated nutrient management technique and by low inputs, plant could promote studied values. It is recommended that further study will be necessary in order to demonstrate the application of such a technique to other crops.

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