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The effects of alfalfa extract and plant growth promoting rhizobacteria on growth and uptake of micronutrients in sorghum (*Sorghum bicolor* L. Var. Speedfeed).

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ABSTRACT

In order to evaluate the effect of alfalfa extract and plant growth promoting rhizobacteria (PGPR) on growth and uptake of micronutrients in sorghum, a pot experiment was arranged in factorial based on completely randomized design with six replications. The first factor was inoculations (non-inoculated-control and inoculated soil medium with PGPR) and second factor included alfalfa extract concentrations (0, 2 and 4 per thousand). The results showed that by increasing the alfalfa extract, dry weight and uptake of micronutrients such as iron, zinc, manganese and copper increased in shoot of sorghum. Adding PGPR to the soil increased uptake of copper in the plant. Also, alfalfa extract at the level of 4 per thousand and PGPR added to growth medium (soil) significantly increased uptake of manganese in the shoot of sorghum. At the level of 2 per thousand of alfalfa extract, adding these microorganisms to the soil significantly decreased dry weight of sorghum shoot. In general addition of the 0.004 alfalfa extract with PGPR in soil had the best effect on studied parameters.

INTRODUCTION

In recent years, human growing demand for animal products due to increasing world population and pastures inability to meet livestock food needs has led cultivation of forage plants have been significantly attended. In this regard, sorghum (*Sorghum bicolor* L.), having features such as high productivity per unit area, high power in paw-handing, high tolerance to drought and good nutritional value, has significant importance; Thus, the development of cultivation of sorghum in arid and semi-arid regions can be effective in providing part of the country's forage requirements (Seyedsharifi and Hokmalipur 2010). Among the factors affecting on plant growth, providing nutrients has important role in developing the quantity and quality of its products. One way to provide nutrients for plants is to use chemical fertilizers. By increasing consumption of chemical fertilizers the world has faced more pollution danger in the environment. Chemical fertilizers used in agriculture have entered in various ways into the groundwater and surface water and even drinking water resources and could threaten human health and the environment (Patton and Crouch 1977). The results of researchers' findings indicated that although consumption of chemical fertilizers could increase crops yield in early years, however, improper usage of those fertilizers have created main adverse effects on environment, biodiversity and cycles of natures over the years (Liu et al. 2006). Thus, today to increase the quantity and quality of produced crops, agricultural experts consider consumption of bio-fertilizers and organic products as an appropriate way in sustainable agriculture that extensive research has been done in this area. Spaepen et al. (2007) and Spaepen et al. (2008) demonstrated that inoculated plants with plant growth promoting rhizobacteria, were success in the uptake of essential elements, resistance against stresses, and rapid growth of plants which were cultivated in the contaminated soils with heavy metals or poor food nutrients. Also in most cases, the use of PGPR improves quantity and quality characteristics of products (Amal et al. 2010; Rahi 2013; Ahmed et al. 2013).

Plant hormones (phytohormones) are other important factors in the growth and development of plants. They are also involved in the response of

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plans to environmental factors. These substances are active in very small quantities and they will be produced in some parts of plant and moved to locations in which cause biochemical, physiological or morphological reactions. Normally plant hormones are auxin, gibberellin, cytokinin, abscisic acid and ethylene (Moor 1989). Some hormones and plants growth promoters are produced and purified industrially. In addition, industrial growth hormones can increase plant growth (Salisbery and Ross 1992). Triacontanol is one of these compounds that was extracted and purified from alfalfa for the first time in 1997 and was implemented as a plant growth stimulant (Ries et al. 1977 a, b). It is part of the vegetable waxes which is a plant growth factor; even Nano-molar concentrations of this substance can increase growth and products productivity (Ries et al. 1977 b). Moreover, it has been used to enhance production of millions of hectares of land in Asia especially in China (Ries et al. 1984; Ries 1991), but in a country like Iran, due to the complexity in the formulation and purification of the compound, and also because of its high costs preparation and application of it, is not common; in this way, alfalfa extract could be used to reach this goal. Shikur (2012) reports are based on beneficial effects of alfalfa extract on the plant growth. Moreover, Ries et al. (1977a) reported that the placement of chopped alfalfa below seeds or seedling location increases dry weight of broccoli seedling in the amount of 36% compared to control. They also indicated that the application of 913 kg chopped alfalfa per hectare enhanced dry weight of tomato 131% than control. Using chopped alfalfa increased stem and root dry weight of corn to 55% and 66%, respectively (Ries et al. 1977a). Ries et al. (1978) reported that 16-days-old rice seedling, treated with a solution of 1g/l crystals isolated from alfalfa, increased nearly 29% total dry weight compared to control. Also, they find that the amount of crystal extracted alfalfa increased dry weight of barely and corn by 22% and 21%, respectively. Because alfalfa is rich in vitamins A, E, C, K and contains considerable quantities of Triacontanol, protein and essential elements for the growth and development of plants (Chopra et al. 1986; Ries et al. 1977 b) so this plant extract can be benefited as fertilizer and plant growth stimulants. With this strategy, farmers can be encouraged to reduce the use of chemical fertilizers and thus prevent environmental pollution.

Despite extensive research in the field of plant nutrition, the effects of both consumption of alfalfa extract and plant growth promoting microorganisms on quantitative and qualitative characteristics of sorghum are not considered. Therefore, it seems that discussion about the effect of alfalfa extract and PGPR on growth and uptake of micronutrients in sorghum is necessary.

MATERIALS AND METHODS

Preparation of cultivation medium

Cultivation medium prepared from 0-15 cm depth of soil in research farm of Zabol University. After air drying and passing through 4mm sieve, it was mixed completely. Then 4 kg of this soil was added to each pot (plastic pots by the size of 3.2 L). 2 kg sample was sent to laboratory to determine physico-chemical properties of soil, which after beaten and passing through 2mm sieve, soil texture by hydrometer method (Day 1982), the percentage of soil organic carbon by oxidation method (Nelson and Sommers 1996), pH in suspension 2:1 water and soil (Thomas 1992), EC in extracts of suspension 2:1 water and soil (Rhoades 1996), available phosphorus with Oslen's extract-consuming method (Olsen and Sommers 1982) by spectrophotometer (Model UV-2100) and available potassium by extract-consuming of acetate ammonium method (Berry et al. 1946) by flamephotometer were measured. Zinc, Iron, Copper and Manganese extracted by ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) and were determined by atomic absorption spectroscopy (Havlin and Sultanpour 1981). Physicochemical properties of soil are shown in Table 1.

Alfalfa extract preparation

To prepare alfalfa extract, 10 kg alfalfa shoot (in bloom) provided and milled after air drying in the shade. Then 50 g milled powder was added to 300 mL ethanol and it was stirred for 24 hours at 20 ˚C on a shaker with 350 rounds per minute. This was purified by Whatman number-one paper (Bahraminejad 2008) and ethanol part was placed under the hood to evaporate and obtain ethanol extract. Then, it was reached to the volume of 300 mL distilled water and stirred well before consumption to obtain homogeneous solution. The concentration of extract elements was measured by using multi-element analysis by ICP-MS Agilent series 4500 (Sobin et al. 2011).

Experimental design and analysis

Pot factorial experiment was arranged in factorial based on completely randomized design with six replications in soil cultivation medium. The first factor was inoculations (non-inoculated-control and inoculated soil medium with PGPR) and second factor included alfalfa extract concentrations (0, 2 and 4 per thousand). Analysis of data was performed by SAS software and means comparison by Duncan's multiple range test at ($p \leq 0.05$).

Table 1. Physicochemical properties of soil

Cu	Mn	Zn	Fe	K	P	OC	EC	pH	silt	clay	sand	Soil texture
of available (mg\kg)						%	ds\m	-	%			-
1.65	5.6	4.8	2.2	430	12	1.98	1.32	7.2	18	13	69	Sandy loam

Table 2. Some measured properties in alfalfa extract

pH	EC	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Ni	Co	Mo
-	ds\m	mg\L											
6.66	0.576	3.2	480	1.6	19.7	4.5	0.1	0.1	1.177	0.98	0.198	0.1	0.025

Plant cultivation and applying treatments

10 sorghum seeds (*Sorghum bicolor* L. var. speedfeed) sown in each pot. After over two weeks, experimental pots were thinned and three plants remained in each pot. Then, 200 mL suspension of PGPR (combination of bio-fertilizers of Azoto Barvar 1 (0.5 g) and Phosphate Barvar 2 (0.5 g) in 3600 mL water) were added to pots' soil and alfalfa extract treatments were applied a week after PGPR inoculation in three stages with intervals of 15 days and concentrations of 0 (control), 2 and 4 per thousand (100 mL per pot). To provide plant growth conditions, day-night temperature were controlled in 33±2 and 25±2 ˚C respectively; and 17 hours photoperiod implemented in greenhouse. Also, soil moisture was kept to 70% **(**by weight**)** of Field Capacity (FC).

Plant harvest and measuring attributes

After passing 75 days of cultivation, the plant shoot was cut from crown and washed with distilled water; Then were dried for 72h at 70˚C (Black and Evans 1965). The dry weight measured by sensitive microbalance (0.001 g). After digestion of plant samples by dry-ashing method (westerman 1990) atomic absorption spectroscopy (UNICAM 919 AA model) was used to determine iron, zinc, manganese and copper concentrations; uptake of these elements measured by following equation.

Element uptake = Element concentration (mg\kg) × Dry matter of sorghum shoot (kg)

RESULTS AND DISCUSSION

Analysis of data variance shows that the main effects of PGPR is significant on dry weight of plant as well as the main effect of alfalfa extract on dry weight and uptake of iron, zinc, manganese and copper at (p≤ 0.01). In addition, main effects of PGPR on copper uptake and interaction between PGPR and alfalfa extract on dry weight and manganese uptake were significant at (p≤ 0.05). (Table 3).

Dry weight

Comparison mean of data shows that, in non-inoculated and inoculated medium with PGPR, increasing alfalfa extract concentration enhanced dry weight of sorghum shoot. In non-inoculated medium, level of 0.004 in comparison with levels of zero (control) and 0.002 of alfalfa extract significantly enhanced dry weight; but levels of control and 0.002 alfalfa extract do not have significant difference in respect to this property. At level of 0.002 alfalfa extract, inoculation of soil with PGPR significantly decreased dry weight of sorghum shoot (Figure 1). Research indicates that use of bio-fertilizers such as Azoto Barvar 1 and Phosphate Barvar 2, improves the quantity and quality of agricultural products in most cases (khalili et al. 2008; Madani et al. 2011; Mohammadi et al. 2012). Mir et al. (2015) reported that any bio-fertilizers application (Azoto Barvar 1 and Phosphate Barvar 2) enhances chlorophyll (a, b), carotenoid and leaf carbohydrates of sorghum. They also stated that combined use of bio-fertilizers to single use of any fertilizers increased this attribute significantly. It should be noted that bio-fertilizers always have no positive effects on the quantity and quality properties of plants and it is likely to have negative and inert effect in some conditions that this depends on nature of life and different behaviors of microorganisms in different conditions. Research results of Nazari et al. (2008) and Abbasnia et al. (2012) confirm this subject about use of bio-fertilizers (phosphate Barvar 2).

Table 3: Variance analysis of PGPR and alfalfa extract effects on dry weight and uptake of micronutrients in sorghum shoot

Mean-square					df	Changes resources
Cu uptake	Mn uptake	Zn uptake	Fe uptake	Dry weight		
0.0005 *	0.0197 ^{ns}	0.0202 ^{ns}	0.0009 ^{ns}	11.6508 **	1	PGPR
0.0080 **	0.9299 **	0.3710 **	2.3654 **	175.0893 **	2	Alfalfa extract
0.0003 ^{ns}	0.0776 *	0.0304 ^{ns}	0.0765 ^{ns}	5.0636 *	2	Alfalfa extract * PGPR
0.0001	0.0164	0.0089	0.0279	1.1145	30	error
11.72	16.53	19.79	14.14	7.31	-	CV

^{ns}, **, * indicate non-significant and significant at (P≤0.01) and (P≤0.05) respectively.

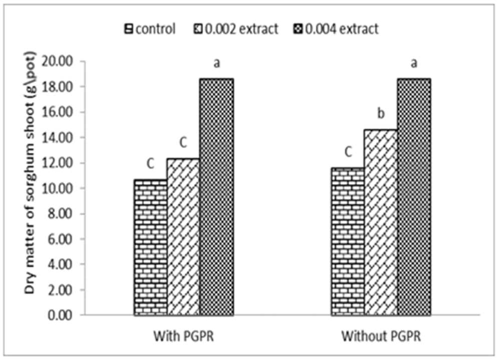

Fig.1. Effect of inoculating soil with PGPR and different levels of alfalfa extract on dry weight of sorghum shoot

According to Table 2, alfalfa extract contain considerable amounts of essential and beneficial nutrients for plants' growth and development. Moreover, this plant extract includes growth-stimulant substance called triacontanol (Ries 1977b). Research shows that triacontanol increase crop yield production of different horticultural and agricultural plants such as asparagus, dry beans, lettuce, onions, tomatoes (Biembaum et al. 1988), carrot, cucumber, corn, melon, glucose (fuel), radish, rice, soybeans and sweet corn (Maugh 1981; Mahadevappa et al. 1989; Prasad and Prasad 1991). In addition, triacontanol increases various parameters related to growth such as dry weight, leaf surface, and root and stem elongation, leaf density, wet and dry biomass (Ericksen et al. 1981; Muthuchelian et al. 2003). Ries et al. (1977b), indicated that triacontanol promotes tobacco callus growth at low concentration (0.001 µg\dish). They also reported that tissue growth development in callus, tomato, potato, barely and beans are reactions to triacontanol concentration (10 µg\dish). Plants growth response is very fast to triacontanol treatment, as far as 3-6 hours after treatments; it is observed that fresh weight of plant increases. Therefore, increasing dry weight of sorghum shoot seems logical by enhancing concentration of used alfalfa extract.

Iron uptake

According to figure 2, results of comparison mean showed that iron uptake in plants shoot significantly increased by enhancing alfalfa extract concentration, so that levels of 0.002 and 0.004 extract in comparison with control indicated 1.7 and 2.3 times an increment in uptake of this element, respectively. Since alfalfa extract contains 0.1 mg iron per liter (Table 2); thus, increasing concentration of consumed alfalfa extract promotes iron in sorghum shoot.

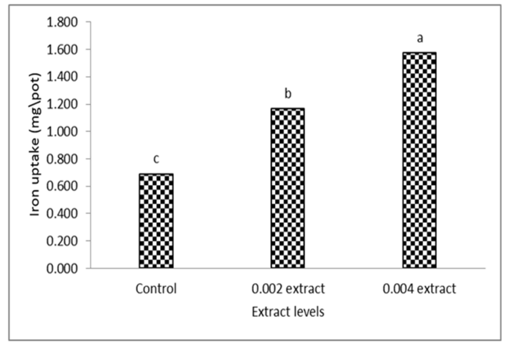

Fig.2. Effect of various levels of alfalfa extract on iron uptake of sorghum shoot

Zinc uptake

The mean comparison shows that uptake of zinc in sorghum shoot significantly enhanced by increasing of alfalfa extract concentration. So that amount of this nutrient increased 2 and 3.3 times at levels of 0.002 and 0.004 alfalfa extract compared to control, respectively (Figure 3). According to Table 2, there is amount of 1.177 mg zinc per liter of alfalfa extract. Therefore, zinc uptake has been also increased in sorghum shoot by increasing concentration of consumed alfalfa extract.

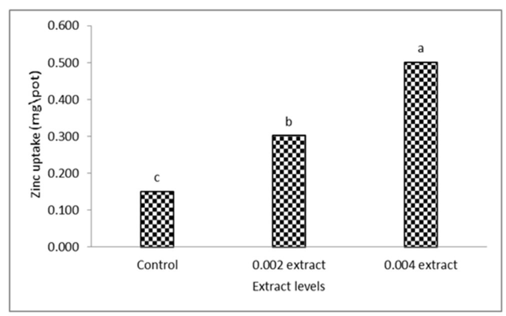

Fig. 3. Effect of different levels of alfalfa extract on zinc uptake of sorghum shoot

Manganese uptake

By considering figure 4, the comparison of mean show that level of 0.004 in compare to control and 0.002 levels of alfalfa extract significantly enhanced manganese uptake in each non-inoculated and inoculated medium with PGPR. Furthermore, non-inoculated medium has no significant difference in using those levels (control and 0.002) compared to inoculated medium with PGPR; However, at 0.004 level of alfalfa extract, inoculated soil with PGPR in comparison with non-inoculated soil, significantly increased this attribute. As regards alfalfa extract contains significant amounts of manganese (Table 2), thereby increasing the concentration of alfalfa extract can increase manganese uptake in plants. Also, it is better to use 0.004 level of alfalfa extract in order to increase significantly manganese uptake in shoot of plants. In most cases, PGPR enhance

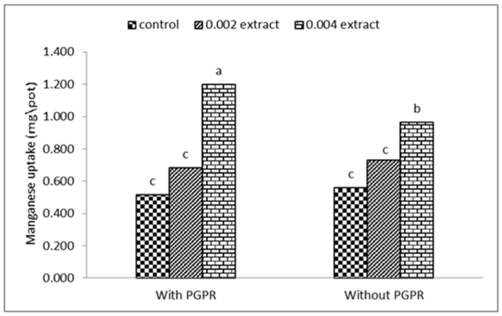

Fig. 4. Effect of inoculating soil with PGPR and different levels of alfalfa extract on manganese uptake of sorghum shoot

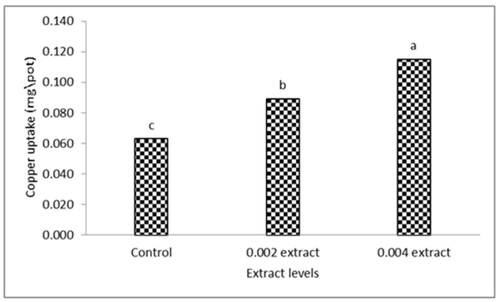

Fig. 5. Effect of different levels of alfalfa extract on copper uptake of sorghum shoot

uptake of essential elements for plants. Dursun et al. (2010) demonstrated that *Pantoea agglomerans* makes significant increment in tomato's manganese. Turan et al. (2014) reported that *Pantoea agglomerans* enhanced 21.7% in manganese concentration of cabbage. Because there was *Pantoea agglomerans* in the composition of used PGPR; Thus, alfalfa extract at level of 0.004 in inoculated soil with PGPR in comparison with non-inoculated soil, significantly enhanced manganese uptake of sorghum shoot.

Copper uptake

By enhancing concentration of alfalfa extract, copper uptake increased in sorghum shoot significantly. In other words, minimum and maximum uptake of copper was obtained at control and 0.004 level of alfalfa extract respectively (Figure 5). Then, copper uptake in sorghum shoot increased 41.3 and 82.5 %, respectively, compared to control at 0.002 and 0.004 levels. With regard to alfalfa extract contains 0.98 mg copper per liter (Table 2), thus increasing the concentration of

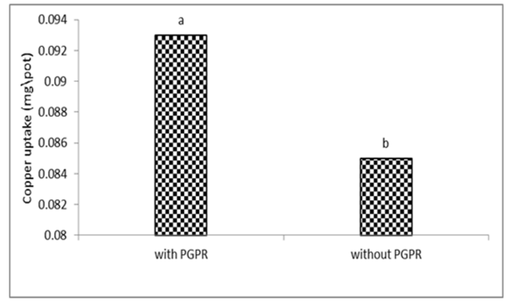

Fig. 6. Effect of inoculating soil with PGPR on copper uptake of sorghum shoot

consumed alfalfa extract causes copper uptake enhances in sorghum shoot as well.

According to Figure 6, results of mean comparison shows that inoculated medium with PGPR compared to non-inoculated medium significantly increased copper uptake of sorghum shoot. Studies indicate that copper uptake has been enhanced by plants in the presence of *Pantoea agglomerans* PGPR (Dursun et al. 2010; Ekinci et al. 2014). Because there was *Pantoea agglomerans* in the composition of used PGPR; then increasing copper uptake of sorghum shoot is justified in inoculated treatments with PGPR.

With regard to Table 4, the exception of non-significant correlation between uptake of copper and zinc, there is a significant and positive correlation between uptakes of iron, zinc, manganese and copper at ($p \leq 0.01$). In addition, there is a positive and significant correlation between dry weight of sorghum shoot with the uptake of iron, zinc, manganese and copper at ($p \leq 0.05$). Because Fe, Zn, Mn and Cu are component of micronutrients for growth and development of plants (Jones 2012). Then, it is reasonable that have positive and significant correlation between dry weights of sorghum shoot and uptake of these elements.

CONCLUSION

This study results showed that alfalfa extract, because of containing significant amounts of essential and beneficial nutrients for plant growth and likely growth stimulants (such as Triacontanol), can increase dry weight and uptake of micronutrients (Fe, Zn, Mn and Cu) in sorghum.

Table 4: the correlation between measured traits.

		Dry weight	Uptake of			
			Iron	Zinc	Manganese	Copper
	Dry weight	1	0/ 8920*	0/ 7206*	0/88020*	0/6686*
Uptake of	Iron		1	0/9372**	0/8158**	0/7147**
	Zinc			1	0/7527**	0/5485^{ns}
	Manganese				1	0/7189**
	Copper					1

^{ns}, **, * indicate non-significant and significant at ($P \leq 0.01$) and ($P \leq 0.05$) respectively.

Also, application of PGPR enhances manganese and copper uptake in sorghum shoot. Generally, alfalfa extract at level of 0.004 with inoculation soil by PGPR is the best treatment for increasing dry weight and uptake of micronutrients of sorghum shoot. The results of this study can be useful to enhance of crop production per unit area. However, further researches require in this matter, especially applied high concentrations of alfalfa extract in interaction with other beneficial microorganisms in field condition.

REFERENCES

Abbasnia Y. Zare S.K. Sedaghathoor S. Padasht D.M.N. (2012) Effect of biofertilizer application on growth parameters of spathiphyllum illusion. American-Eurasian Journal of Agricultural and Environmental Sciences, 12 (5): 669-673.

Ahmed S.H. Gendy A.H. Hussein A.A.M. Said-Al A.H.L. Mohamed H.F.Y. (2013) Effect of some nitrogen sources, bio-fertilizers and their interaction on the growth, seed yield and chemical composition of guar plants. Life Science Journal, 10(3): 389-402.

Amal G.A. Orabi S. Gomaa A.M. (2010) Bio-organic farming of grain sorghum and its effect on growth, physiological and yield parameters and antioxidant enzymes activity. Research Journal of Agriculture and Biological Sciences, 6(3): 270-279.

Bahraminejad S. Asenstorfer R.E. Riley I.T. Schultz C.J. (2008) Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots oats (*Avena sativa* L.). Journal of Phytopathology, 156: 1-7.

Berry J.W. Chappell D.G. Barnes R.B. (1946) Imporved method of flame photometry. Industrial and Engineering Chemistry, Analytical Edition, 18 (1): 19-24.

Biembaum J.A. Houtz R.L. Ries S.K. (1988) Field study with crops treated with colloidally dispersed triacontanol. Journal of the American Society for Horticultural Science. 113(5): 679-684.

Black C.A. Evans D.D. White J.L. Ensminger L.E. Clark F.E. (1965) Methods of Soil Chemical Analysis, Part 2. American Society of Agronomy. Madison, WI.

Chopra N. Nayar L. Chopra C. (1986) Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi.

Day, P. R. (1982) In Methods of soil Analysis, Part 2; Page A.L. et al. edi. Agronomy Monograph 91, American Society of Agronomy. Madison, WI.

Dursun A. Ekinci M. Figen D.M. (2010) Effects of foliar application of plant growth promoting bacterium on chemical contents, yield and growth of tomato (*Lycopersicon esculentum* L.) and cucumber (*Cucumis sativus* L.). Pakistan Journal of Botany, 42(5): 3349-3356.

Ekinci M. Turan M. Yildirim E. Güneş A. Kotan R. Dursun A. (2014) Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone concentration of cauliflower (*Brassica oleracea* L. var. botrytis) transplants. Acta Scientiarum Polonorum-Hortorum Cultus, 13(6): 71-85.

Ericksen A.B. Sellden G. Skogen D. Nilssen S. (1981) Comparative analysis of the effect of triacontanol on photosynthesis, photorespiration and growth of tomato (C3 plant) and maize (C4-plant). Planta, 152: 44-49.

Havlin J.L. Sultanpour P.N. (1981) Evaluation of the AB-DTPA soil test for iron and zinc. Soil Science Society of America Journal, 45: 55-70.

Jones J. B. 2012. Plant Nutrition and Soil Fertility Manual, Second Edition. CRC Press, US.

Khalili A. Akbari N. Chaichi M.R. (2008) Limited irrigation and phosphorus fertilizer effects on yield and yield components of grain sorghum (*Sorghum bicolor* L. var. Kimia). American-Eurasian Journal of Agricultural and Environmental Science, 3(5): 697-702.

Liu X. Feng Z. Zhang S. Zhang J. Xiao Q. Wang Y. (2006) Preparation and testing of cementing nano-subnano composites of slowor controlled release of fertilizers. Scientia Agricultura Sinica, 39: 1598-1604.

Madani H. Malboobi M.A. Bakhshkelarestaghi K. Stoklosa A. (2011) Biological and chemical phosphorus fertilizers effect on yield and P accumulation in rapeseed (*Brassica napus* L.) Notulae Botanicae Horti Agrobotanici. 40(2): 210-214.

Mahadevappa M. Nurthy R.A. Biradar B.B. (1989) Effect of triacontanol on rice seedlings weight and gain yield. International Rice Research Center News Letter, 14: 26-29.

Maugh T.h. (1981) New chemicals promise larger crops: synthetic analogs of brassinolide prove very effective, while the mystry of triacontanol's inconsistency may finally have been solved. Science, 212: 33-34.

Mir S. Sirousmehr A. Shirmohammadi E. (2015) Effect of nano and biological fertilizers on carbohydrate and chlorophyll content of forage sorghum (Speedfeed hybrid). International Journal of Biosciences. 6 (4): 157-164.

Mohammadi G.R. Eghbal G.M. Sheikheh-Poor S. (2012) Phosphate Biofertilizer, Row Spacing and Plant Density Effects on Corn (Zea mays L.) Yield and Weed Growth. American Journal of Plant Sciences, 3: 425-429.

Moore T.C. (1989) Biochemistry and Physiology of Plant Hormones. Springer Verlag New York.

Muthuchelian K. Velayutham M. Nedunchezhian N. (2003) Ameliorating effect of Triacontanol on acidic mist-treated Erythrina variegate seedlings changes in growth and photosynthetic activities. Plant Science, 165: 1253-1257.

Nazari F. Farahmand H. Eshghi S. Niki M. Eslamzade M. (2008) The effect of different soil amendments on growth and flowering of African marigold (*Tagetes erecta* L.) 'Queen'. Journal of Fruit and Ornamental Plant Research. 16: 403-415.

Nelson D.W. Sommers L.E. (1996) Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 2, 2nd ed., A.L. Page et al., Ed. Agronomy. 9:961-1010. American Society of Agronomy. Madison, WI.

Olsen S.R. Sommers L.E. (1982) Phosphorus. P 403–427, In: A.L. Page et al. (ed.), Methods of soil analysis, Part 2. 2nd ed. Agron. Monogr, ASA and Soil Science Society of America, Madison, Wisconsin.

Patton C. Crouch S. (1977) Determination of serum urea enzymatically. Analytical Chemistry, 49: 464-469.

Prasad M. Prasad R. (1991) Effect of triacontanol on rice growth and yield. Indian Journal of Agronomy, 36: 75- 77.

Rahi AR. (2013) Effect of nitroxin biofertilizer on morphological and physiological traits of *Amaranthus retroflexus*. Iranian Journal of Plant Physiolgy, 4(1): 899-905.

Rhoades J.D. (1996) Salinity: electrical conductivity and total dissolved solids. P 417-437, In: D.L. Sparks (ed.), Methods of Soil Analysis, Part 3 chemical methods, SSSA, Madison, Wisconsin.

Ries S.K. (1991) Triacontanol and its secondary messenger 9-Adenosine as plant growth substances. Plant Physiology, 95: 986-989.

Ries S.K. Bittenbender H. Hangarter R. Kolker L. Morris G. Wert V. (1977a) Improved Growth and Yield of Crops from Organic Supplements. In: Energy and Agriculture Academic Press, New York.

Ries S.K. Richman T.L. Wert V.F. (1978) Growth and yield of crops treated with triacontanol. Journal of the American Society for Horticultural Science, 103: 361-364.

Ries S.K. Wert V. Biernbaum J.A. (1984) Interference of Triacontanol activity by chemical constituents present in experimental and field sprayers. Journal of the American Society for Horticultural Science, 109: 145-150.

Ries S.K. Wert V.F. Sweeley C.C. Leavitt R.A. (1977b) Triacontanol: a new naturally occurring plant growth regulator. Science, 195: 1339-41.

Salisbery f.B. Ross C.W. (1992) Plant Physiology (4th ed) wadsworth California.

Seyedsharifi R. Hokmalipur S. (2010) Forage Plants Cultivation. Mohaghegh Ardabili University and Amidie Tabriz publishers.

Shikur T.K. (2012) Alfalfa extract as a plant growth regulator: Response of Troyer Citrange (*Citrus Sinensis* X *Poncirus Trifoliata*) seedlings to Alfalfa (*Medicago Sativa* L.) crude extract. LAP LAMBERT Academic Publishing.

Sobin C. Parisi N. Schaub T. Altman B. (2011) Comparison of the lead care system and inductively coupled plasma mass spectrometry for detecting low-level lead in child whole blood samples. Journal of Medical Toxicology, 7: 24-32.

Spaepen S. Dobbelaere S. Croonenborghs A. Vanderleyden J. (2008) Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. Plant and Soil, 312: 15-23.

Spaepen S. Vanderleyden J. Remans R. (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31: 425-448.

Thomas G.W. (1996) Soil pH and soil acidity. P 475-491, In: D.L. Sparks (ed.), Methods of Soil Analysis, Part 3 chemical methods, Soil Science Society of America, Madison, Wisconsin.

Turan M. Ekinci M. Yildirim E. Gunes A. Karagoz K. Kotan R. Dursun A. (2014) Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. Turkish Journal of Agriculture and Forestry, 38: 327-333.

Westerman L.R. (1990) Soil Testing and Plant Analysis. Soil Science Society of America, INC. Madison, Wisconsin, USA.

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