Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2012, 2 (4):1326-1336

ISSN: 2248 –9215
CODEN (USA): EJEBAU

The effect of Fertilizers and biological nitrogen and planting density on yield quality and quantity *Pimpinella anisum* L.

E. Nabizadeh[1], H. Habibi[2] and M.Hosainpour[3]

[1]*Faculty of Agriculture and Resource, Islamic Azad University, Mahabad branch, Iran*
[2]*Dept. of Agronomy and Plant Breeding, Faculty of Agriculture, Shahed, Iran*
[3]*Department of Agronomy, Shahed University, Iran*

ABSTRACT

Considering the importance of medicinal plants growth and biological application of fertilizers with sustainable agricultural production in order to eliminate or reduce chemical input to achieve desirable and sustainable quality, an experimental research based on randomized complete block design with 3 factors of chemical nitrogen (46% urea nitrogen) at 3 levels (Zero, 60 and 120 kg/ha), biological nitrogen (Azotobacter) with trade name Nitroxin at 3 levels (Zero, 3 and 6 lit/ha) with 3 plant densities (with fixed rows of 30 cm, with plant spacing's on the row 2, 4, 8 cm equivalent to 50, 25 and 12.5 plant/ m^2) was carried in 2009. During the plant growth the necessary samplins were taken and the physiological indices of growth were measured. The results of analysis of variance showed that the effects of biological fertilizers (Azotobacter) Nitroxin of chemical (urea 46%) nitrogen with plant density in different treatments on plant height, leaf number, peduncle length, fertile umbellate/umbel, seed yield, essential oil content and essential oil yield were significant at $p \leq 1$ and 5%. The means showed that the greatest plant height and peduncle length belonged to the density of 50 plant/m^2 (respectively with 48.2 and 5.3 cm). Highest number of leaves (the plant number to the number 19.1) was related to the interaction between biological fertilizer at 6 lit Nitroxin/ha and b density of 25 plant/ m^2. The most seed yield and the highest oil were achieved (1286.4 and 179.1 kg/ha) by fertilizer treatment from interaction between biological Nitroxin (3lit/ha) and chemical nitrogen at 60 ha/kg with the density of 25 plant/ m^2 respectively.

Key Words: Biological nitrogen, chemical nitrogen, Nitroxin, physiological indicators of growth, *Pimpinella anisum,* planting distance.

INTRODUCTION

Anise (*Pimpinella anisum* L.) is one of the most important annual medicinal plant in the world and is active substances are used in various pharmaceutical and food industries. Herbaceous plant anise is, aromatic and Umbelliferae (Apiaceae). The seeds are the plant major organ to producing the main essential oil. Fertilizers have played an effective role in increasing crop yield, and the indiscriminate use of chemical fertilizers can cause environmental and, human health problems depletion of, non-renewable resource, makes plants less resistant to pests and diseases [1, 2, and 3]. The biological advantages compared to chemical fertilizers including the remarkable stability of the cycle of nutrient elements [4], and from of economic and environmental point of view are accepted [5, 6].

Today the use of beneficial soil organisms called biological fertilizers as the most natural and desirable solution for keeping the soil alive and active in the soil the vital agricultural land is considered [7]. Biological fertilizers

(Azotobacter) absorb and increasing the concentration of essential elements such as nitrogen, phosphorus, potassium, zinc, magnesium, iron and protein in crops [8]. Research has shown that the performance and the ability of Azotobacter in nitrogen fixation and balance in the soil depend on the soil properties and plant [9]. Despite the significant positive effects of Azotobacter on plants, the it's exact function in the development of plant growth is still unknown. Pereira et al. [10] in their studies on inoculated pearl millet announced the increased performance by more than 33 percent. Research has shown that the effect of biological Azatobacter fertilizer was significant on pepper, and the highest pepper yield was reported 3.34 ton/ha [11]. Nitrogen improved the performance of more than 30 percent of Geranium (Pelargonium) and other medicinal plants [12]. Application of 100 kg/ha of nitrogen increased the production of secondary metabolites (cisque-Terpin) and percentage *Chamomile* (Matricaria chamomilla) increased dry matter from 3 to 6 percent [13]. Also effect of nitrogen on dry weight and percent Thymus kotschyanus species was significant but left no significant impact on the amount and percentage of oil and carvacrol [8]. Research showed that the amount of nitrogen up to 120 kg /ha produced more thymol yield in thyme oil, but had no significant effect on the amount of seed oil [14]. Nitrogen increased thymus vulgaris oil yield and percent thymol and the best treatment was 100 kg N/ha [15]. Research showed that by increasing Nitrogen application from 105 to 120 kg/ha the essence yield and thymol increased significantly, but had no significant effect on the amount of seed oil [14]. Setting the distance or population of plants is a powerful tool to control plant competition in a species to produce the most active substance [16], the plant density or production rates as a factor in control are important and the basic principles of Agriculture of any product is to identify the appropriate plant density [17, 18]. Cultivation of medicinal plants and plant density on the amount of nutrient elements can affect the quality of essential oils [19]. Considering the importance of nitrogen in plant growth and positive impact on the health of biological fertilizer production of plants, as well as high cultivation of anise in Iran, therefore study biological effect of nitrogen fertilizer on yield quality and quantity of this herb is necessary.

MATERIALS AND METHODS

This case study was performed in agricultural experimental farm located in West Azarbaijan Province in 2010 Mahabad city (35 ° 58 N and 44 ° 3 E of 1354 meters above sea level. The climate of the locations was semi-arid region, 354 mm (mean annual precipitation) with medium heavy soil texture (silty-clay). The field was prepared in autumn and in February the crop was planted. The study is arranged in a factorial experiment with 3 factors and 3 levels of chemical nitrogen (0, 60, 120 kg/ ha), biological nitrogen Nitroxin (0, 3, 6, Lit /ha) and plant density in three levels (2, 4 and 8 cm) based on randomized complete blocks design with three replications. Experimental units with the 3×1.5 m dimensions, rows were 30 cm apart and 3 m long. The anise seeds were planted distance were 1 cm apart, covered with wet sand and about a centimeter thick and after emerging from the soil, thinning operation to set the desired density was performed.

Biological nitrogen fertilizer (Nitroxin) solution was applied as sprinkling system. Three-quarters of fertilizer was applied at planting seeds and the rest was applied to plant at shooting. Basin irrigation until harvest was done depending on weather conditions and weeds were controlled. In order to measurement of characteristics of effective on yield components and substance effective, after removing the marginal effects of each plot, 10 plants from each plot were harvested randomly.

Oil percentage in air-dried herb
The volatile oil from air-dried herb of marjoram plant was isolated by water distillation, using 25 g dried herb according to Balbaa et al. [20], while essential oil yield/plant (ml/100 g) was determined according to Güenther [21]. The oil percentage (%) of plant dry weight (air dried) and the estimated essential oil yield/ ha was thus calculated.
The data were analyzed using statistical program. Means were compared by Duncan's multiple range tests at 0.01 and 0.05 probability level for all comparisons.

RESULTS AND DISCUSSION

According to the ANOVA table, the impact of chemical nitrogen fertilizer and plant density Nitroxin biological interactions between them on the number of branches, number umbellete per plant, seed number per umbel, inflorescence length, percentage of seed weight and oil volume was not significant (Table 1).

Plant height
The results showed that levels of urea nitrogen and Nitroxin (Azotobacter) and interaction effects between the plant density and the fertilizer on the plant height there is no significant difference. But the effect of plant density on plant height level was significant ($P<0.05$) (Table 1). Comparison of density effects on plant height showed that densities of 25 and 50 plants /m^2 respectively, 47.6 and 48.2 cm and the highest density of 12.5 plants m 45.3 cm had the

lowest height (Figure 1). Research showed that nitrogen increased plant height of basil Fenugreek [16] and plant height had a significant effect on Thymus herb [22].

Total leaf

Based on the results of this study the effect of chemical nitrogen and biological fertilized and plant density on the number of leaf were not significant different, but the interaction between biological fertilizers and plant density on the number of leaves was significant at (P <0.05) (Table 1). Comparison showed that the interaction of biological fertilizer rate of 6 liters Nitroxin acre density 25 plants per square meter number of nineteenth and leaves the highest biological treatment fertilizer interaction Nitroxin (control) with a density of 12.5 plants square meters total 17.7 leaves had the lowest number of leaves (Figure 2). In this regard, Hormuz [23] reported that the effect of nitrogen and gibberellic acid on yield, number of leaves and lavender active Common valerian (Valeriana officinalis) was significant.

Flower tail length

There was no significant difference between chemical nitrogen Nitroxin and their interaction, but the effects of density on the tail length were significant (P <0.05), (Table 1). Comparison shows that the density of 25 and 50 plants/ m^2 had the greatest flower tail 5.5 and 5.34, respectively and the density of 12.5 plants /m^2 had the least flower tail of 4.98 (Figure 3).

Total umbellate in each umbel

The analysis of variance showed that the density and chemical nitrogen had no significant effect on umbellate per umbel, but the effects of Nitroxin on the number of umbellates were significantly different at P <0.05 (Table 1) . Mean comparison showed that the fertilizer Nitroxin 3 Lit/ha had the highest umbellate per umbel (13.1) and the control treatment (no fertilizer) had the least umbellate per umbel (12.2) (Figure 4). In this case, the positive impact of some sources of biological fertilizers on the growth of herb garden thymus [24], plant species of wild Thymuse [22], Rosmary [25] was reported. Moraghabi et al. [26] reported that the effect of nitrogen fertilizer reduced the number of umbrellas. Rassam et al. [27] has reported in low density the number of umbel of anise increased. Results of Shareh and Rashed Mohassel [28] showed that yield increased with increasing plant population and led to reduction of number of lateral branches per plant and number of umbrella.

Grain yield

The results of the analysis variance showed that the grain yield was significantly affected by all treatments (P≤0.01) in this experiment (Table 1). Mean comparison table showed that the highest yield (1286.4 kg/ha) belonged to the treatment Nitroxin (3li/ha) + chemical nitrogen (60 kg/ha) with plant density of 25 plants/m^2.

In general, with increasing vegetative growth the nitrogen showed it is effect in increasing the grain yield but should be noted that the increasing in nitrogen shouldn't go beyond the optimum threshold. The significance of the interaction is indicative of plants sensitivity to the studied factors and their synergic effect.
The results of this study conform to the findings of Rassam [27] and Yadava [29].

Essence Yield

Table 1 showed that the effect of each treatment including Nitroxin, chemical nitrogen and plant population alone and as a compound was significantly different on the Essence yield of Essence plant at p≤0.01. Mean comparison showed that the highest Essence yield belonged to the compound of (Nitroxin + chemical nitrogen) at plant density of 25 plant/m^2 (Figure 6). The amount of produced essence in relationship with plant population. Although the effective elements of plants are produced by genetic processes but their production is affected by different factors such as: yield loss, wrong management and particularly nutrients deficit [30]. Because the above mentioned factors cause changes in growth and development and quality and quantity of effective elements (essence) of medicinal plants (such as alkaloids, glycosides', steroids and essences) [31].

The results of the present study confirm with the results of Azizi [32] reporting the effect of nitrogen on the essence yield in anis plant.

Shalaby and Razin [33] reported that application of 105 kg/ha of nitrogen increased essence and thymul in Thymus plant.

Table 1: Results of variance analysis and biological effects of chemical fertilizers (Azotobacter) and plant density on yield quality and quantity anise

Mean of squares (MS)																	
Harvest index oil	Seed harvest index	Biological yield	Seed weight	Essential function	Percent of oil volume	Seed yield	Chlorophyll	Number seed in umbrella	number umbellate in umbrella	Number Umbrella plant	Peduncle length	Inflorescence length	Number of branches	Number leaves	Plant height	df	Resource changes
2.001**	0.693**	22701.778**	^{ns} 3.418	3254.469**	61.355**	2934.909**	^{ns} 60.718	5.776*	1.040^{ns}	53.285^{ns}	3.824^{ns}	0.281^{ns}	10.843^{ns}	2.614^{ns}	37.549^{ns}	2	Repetition
0.654**	32.693**	395610.37**	^{ns} 0.242	5548.396**	1.061^{ns}	301438.471**	^{ns} 60.718	0.696^{ns}	6.160*	9.754^{ns}	1.120^{ns}	10.010^{ns}	1.231^{ns}	1.966^{ns}	7.947^{ns}	2	Nitroxin
1.161**	46.657**	297552.148**	^{ns} 0.156	2498.845**	2.312^{ns}	116961.806**	^{ns} 61.157	0.601^{ns}	2.753^{ns}	31.290^{ns}	0.614^{ns}	3.864^{ns}	8.778^{ns}	2.160^{ns}	6. 961^{ns}	2	Nitrogen
0.481**	6.246**	313396.13**	^{ns} 0.278	877.186**	4.731^{ns}	30787.272**	^{ns} 53.291	2.565^{ns}	0.924^{ns}	18.165^{ns}	2.913^{ns}	19.481^{ns}	1.620^{ns}	1. 133^{ns}	14.539^{ns}	4	Nitroxin× Nitrogen
14.95**	894.245**	91494615.111**	^{ns} 0.022	16269.614**	1.808^{ns}	854362.754**	^{ns} 52.647	0.969^{ns}	0.466^{ns}	38.356^{ns}	4.372*	21.410^{ns}	1.778^{ns}	0.929^{ns}	63.444 *	2	Density
^{ns} 0.186	12.943**	885038.981**	^{ns} 0.223	1490.536**	3.592^{ns}	115277.415**	^{ns} 76.240	1.602^{ns}	1.290^{ns}	1.012^{ns}	0.134^{ns}	51.406^{ns}	7.245^{ns}	2.957*	18.363^{ns}	4	Nitrogen× Density
0.289^{ns}	13.296**	101425.593**	^{ns} 0.104	624.505**	3.051^{ns}	31407.114**	^{ns} 54.732	1.647^{ns}	2.827^{ns}	8.362^{ns}	0.606^{ns}	17.000^{ns}	4.444^{ns}	0.373^{ns}	5.738^{ns}	4	Nitrogen× Density
0.636**	17.109**	83138.769**	^{ns} 0.049	636.919**	2.69^{ns}	16822.049**	^{ns} 49.700	1.163^{ns}	1.054^{ns}	19.343^{ns}	1.221^{ns}	8.386^{ns}	5.634^{ns}	1.908^{ns}	26.197^{ns}	8	Nitrogen×Nitrogen×Density
0.131	0.73	25091.714	0.162	162.268	3.27	380.879	44.779	1.571	1.688	24.459	1.334	22.304	3.987	1.034	17.263	52	Error

*ns **, * respectively; no significant meaningful level of 5% and 1%*

Biological Yield

Biological yield of Anis was significantly affected by chemical nitrogen, plant population and the interaction between them (Table 1). Figure 7 shows that the highest biological yield belongs to the treatment Nitroxin (3lit/ha) with plant population of 50 plant /m^2. At high plant densities competition among plants of the same species usually leads to decrease in single-plant. But increase in plant population compensates for weight loss and biological yield increased with increasing plant population.

Essence and seed harvest index

Results of analysis of variance showed the treatments alone and together had significant affect on the essence and harvest index at $P \leq 0.01$.

The means comparison showed that the compound treatment, Niroxin (3lit/ha) + chemical nirogen (60 lit/ha) at plant density of 25 plant/m^2, had the greatest effect on the essence and harvest index (Figures 8 and 9).

Based on definition, all factors affecting economic yield (like capitol, essence and grain) and total dry matter weight also affect harvest index severely. The results of the present study conform to those of Karimi and Azizi [34] in medicinal plants.

The noticeable point is that the effects of treatments (Nitroxin + plant density) and (Nitrogen + plant density) weren't significantly different on the essence harvest index that in this regard more studies should be done in future. Because the aim of planting medicinal plants is to use their essence.

Table 2: Comparison of effects of chemical and biological fertilizers (Azotobacter) and plant density on morphological traits, medicinal plants anise.

Peduncle length(cm)	Inflorescence length(cm)	number umbellate in umbrella(The number of plant)	Number of branches (The number of plant)	Number leaves (The number of plant)	Plant height (cm)	Adjective Treatments
5.2	28.2	12.17 [b]	8.9	18.4	46.6	Nitrogen control (zero L ha)
4.8	27.2	13.09 [a]	9.3	18.2	46.9	3
5.2	27.1	12.43 [ab]	9.1	18.7	47.6	6
4.9	27.3	12.28	8.9	18.7	47.4	Chemical control Nitrogen (zero kg ha)
4.9	27.2	12.91	8.6	18.1	46.5	60
5.2	27.9	12.50	9.8	18.5	47.3	120
5.3 [a]	28.3	12.28	9.3	18.4	48.2 [a]	Density 50 p. m^2
5.3 [a]	27.5	12.91	9.3	18.6	47.6 [a]	25
4.6 [b]	26.6	12.50	8.8	18.2	45.3 [b]	12.5

Similar letters indicate no significant differences between treatments are average. Analysis of variance for traits that were significantly associated with surgery was not performed to compare mean.

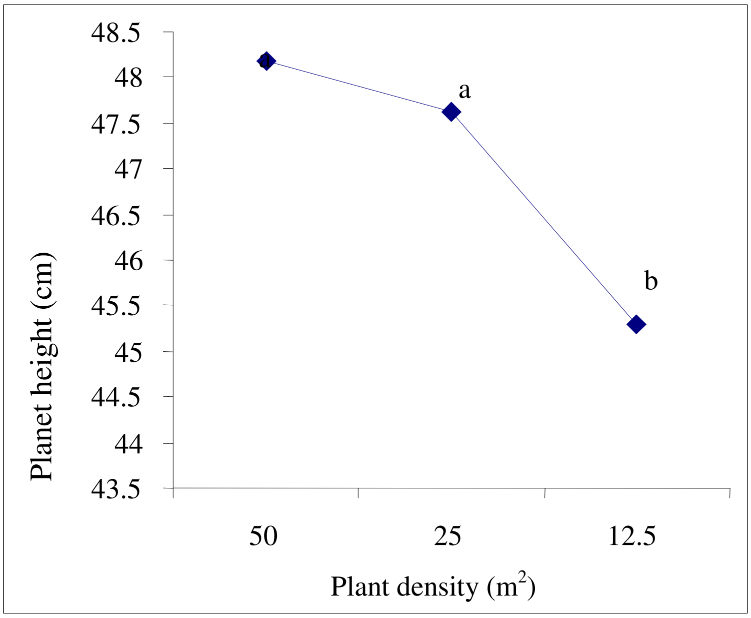

Figure 1: Effect of plant density on plant height, medicinal plant anise.

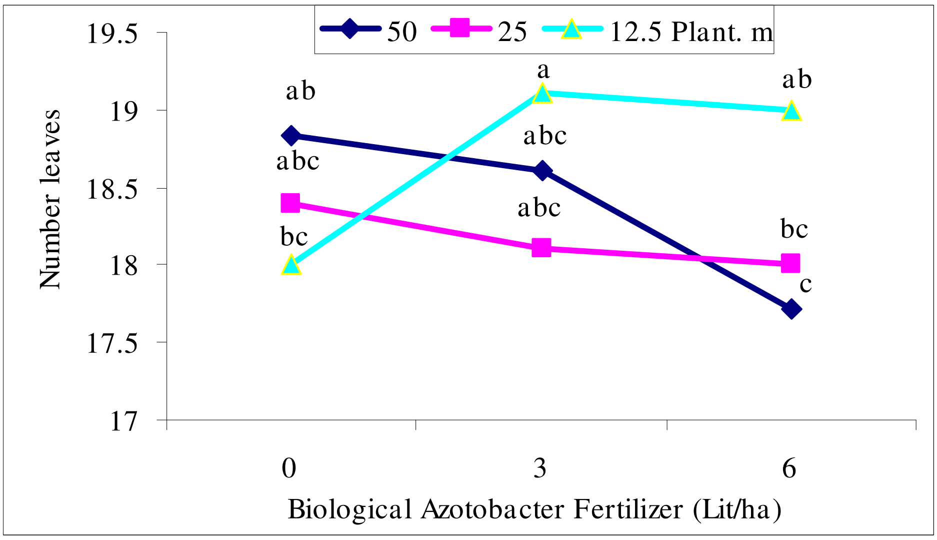

Figure 2: Interaction between Biological Azotobacter Fertilizer plant density on the number of leaves with medicinal plants anise.

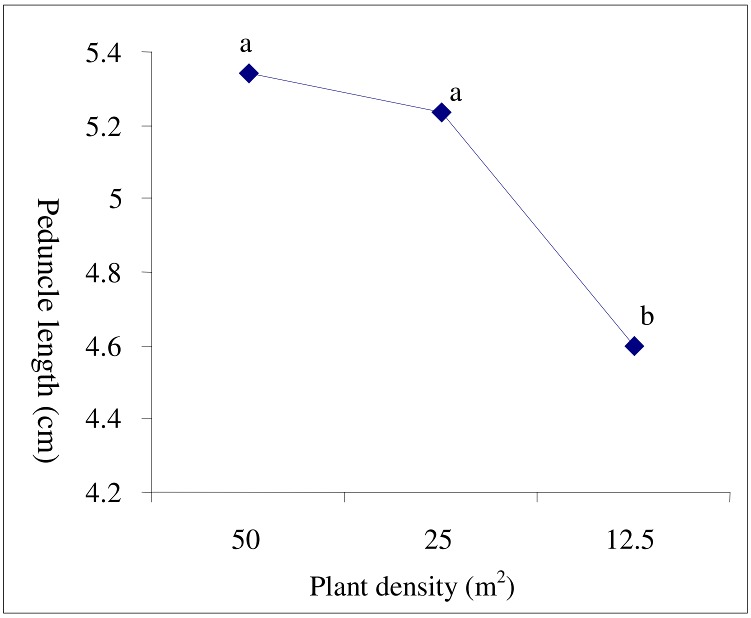

Figure 3: Effect of plant density on peduncle length medicinal plants anise.

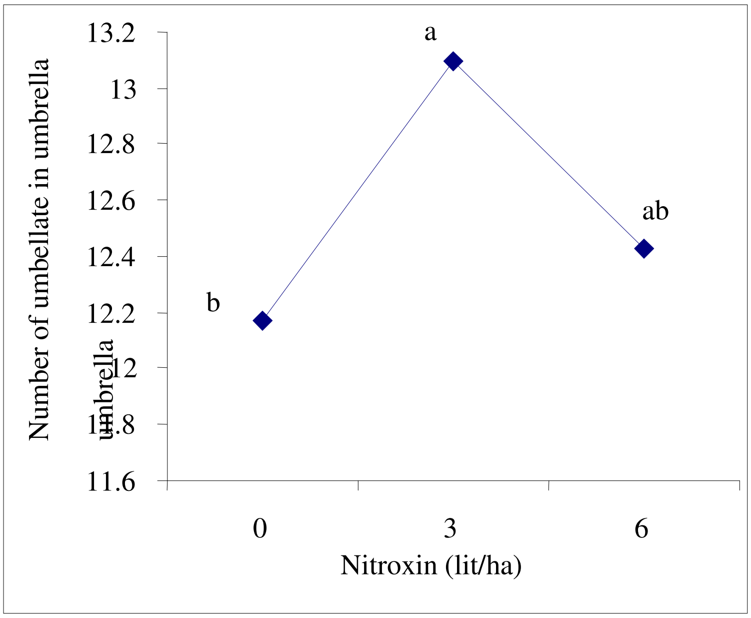

Figure 4: Effect of bio-fertilizers on the number of umbellate in umbrella medicinal plant anise.

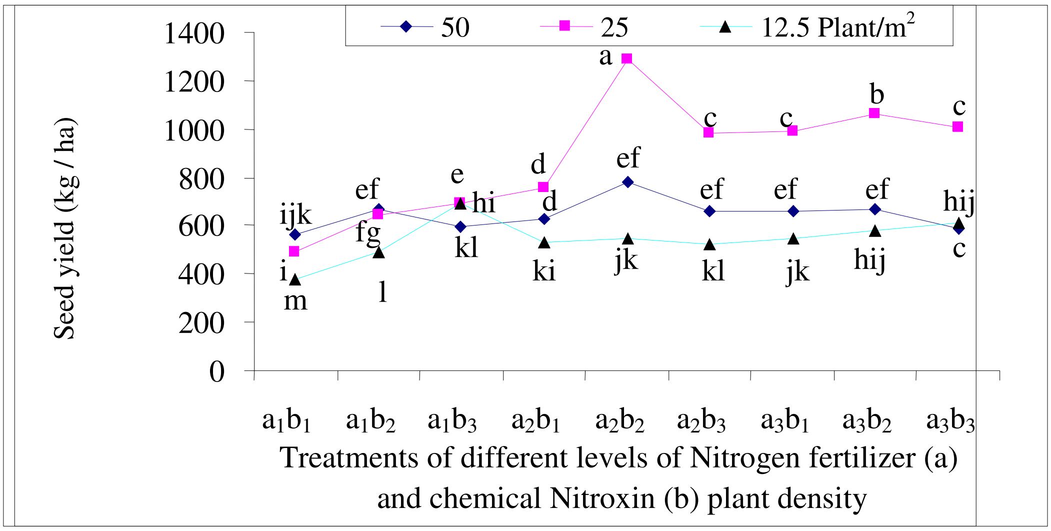

Figure 5: Interaction between different levels of nitrogen fertilizers, chemical, biological plant density on yield medicinal plants anise.

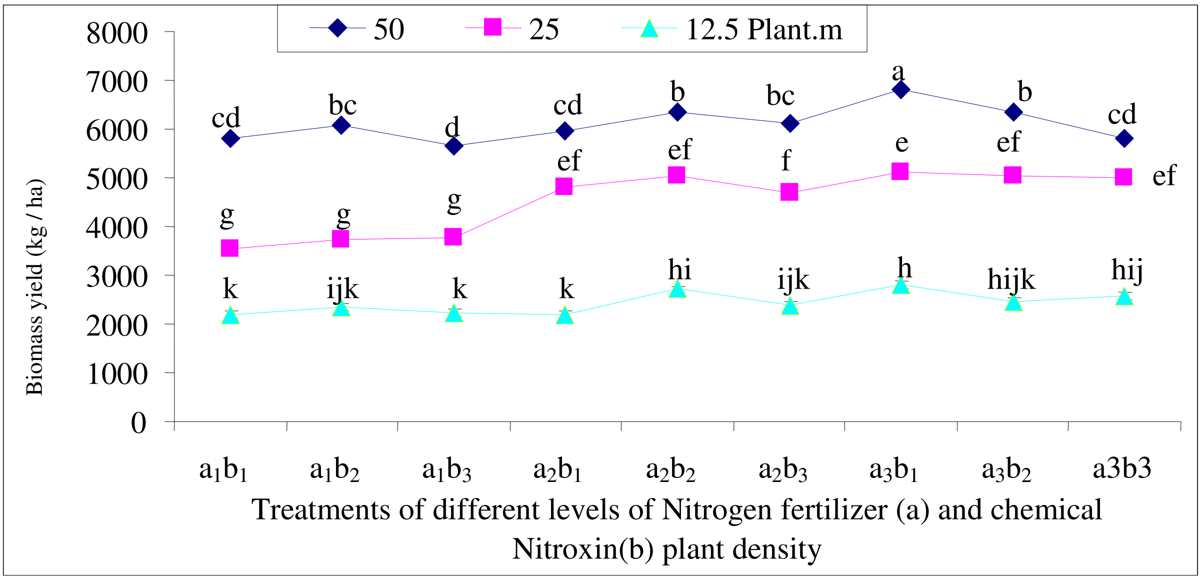

Figure 6: Interaction between different levels of nitrogen fertilizers, chemical, biological With plant density on essential oil yield medicinal plants anise oil.

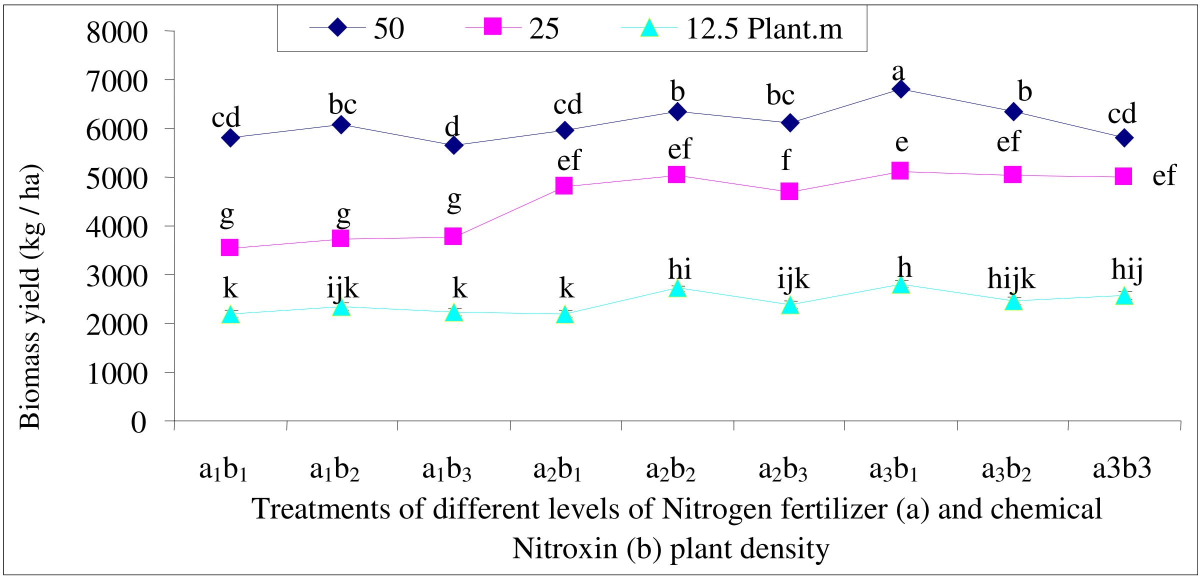

Figure 7: Interaction between different levels of nitrogen fertilizers, chemical, biological With plant density on yield anise plant biomass.

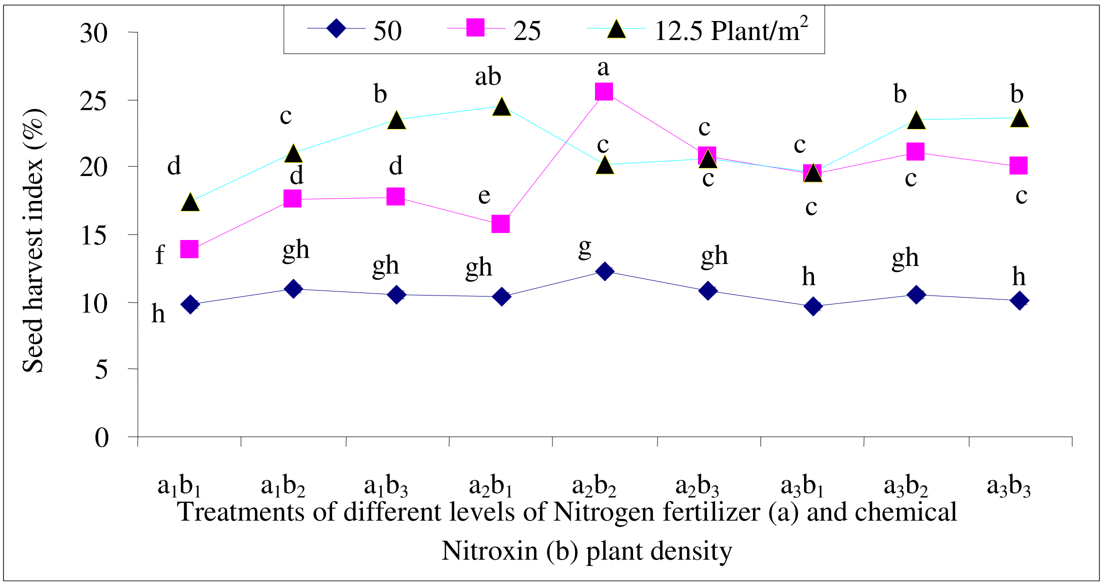

Figure 8: Interaction between different levels of nitrogen fertilizers, chemical, biological plant density on essential oil yield medicinal plant anise.

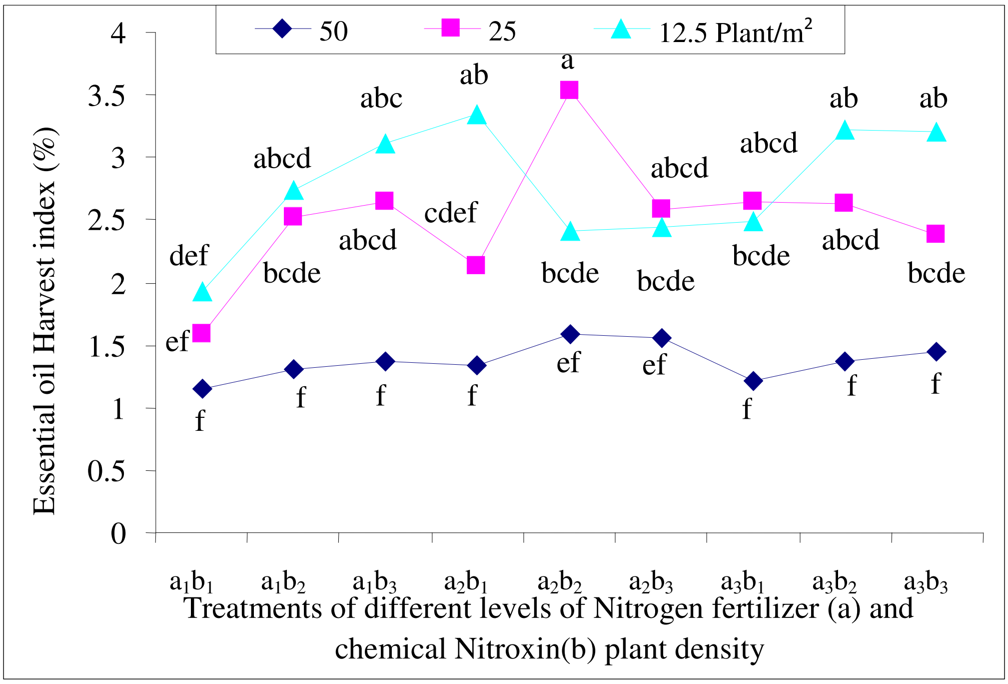

Figure 9: Interaction between different levels of nitrogen fertilizers, chemical, biological with plant density on harvest index essential oil medicinal plants anise.

REFERENCES

[1] K. Brandt, Plant health, Soil fertility relationships and food quality, Proceeding of Organic Agriculture in Aisa, 13-14 March 2008, Seoul, Korea, pp 18-30, **2008**.
[2] A. K. Sharma, Biofertilizers for sustainable agriculture a handbook of organic farming, Agrobios, India, **2002** b.
[3] S. S. Shivaputra, C. P. Patil, G. S. K. Swamy, P. B. Patil, Effect of vesicular-arbuscular mycorrhiza fungi and vermicompost on drought tolerance in papaya. Mycorrhiza News, **2004**, 16 (3), 12-13.
[4] A. Mohallem, H. Heshghizadeh, Application of bio-fertilizers: privileges and limitations, Proceedings of the National Conference of Ecology Iran, Gorgan, pp 47, **2007**.
[5] S. C. Wu, Z. H. Cao, Z. G. Li, K. C. Cheung, M. H. Wong, *Geoderma*., **2005**, 125, 155-166.
[6] J. Fallahi, H. R. Kochaki, P. Rezvani Moghaddam, *Iranian Journal of agricultural Research*., **2009**, 7 (1), 135-127.
[7] N. Saleh Rastin, Biological fertilizers and their role in order to achieve sustainable agriculture, Proceedings of the necessity of manufacturing bio-fertilizers in the country, pp 54, **2001**.
[8] R. Habibi, A. Farahani, H. Habibi, Message Research - Scientific Calendar - Applied Research Center, Agriculture and Natural Resources of Tehran., **2004**, 7, 17.
[9] N. Requena, T. M. Baca, R. Azcon, *Biol. Fert. Soils*., **1997**, 24, 59-65.
[10] P. A. R. Pereira, V. A. Cavalcante, J. I. Baldani, J. Dobereiner, *Plant Soil*., **1998**, 110, 269-274.
[11] J. Mandel, P. Ghanti, B. Mahato, A. R. Mondal, U. Thapa, *Environ Ecol*., **2003**, 21 (3), 712-715.
[12] E. V. S. P. Rao, M. Singh, R. S. Ganesha Rao, *Int. J. Trop. Agric*., **1998**, 6, 95-101.
[13] J. Bullock, Proposal for gaining information on producing tanacetum (fever few) as a high dollar perennial crop, North Carolina state university publication, pp 10, **1999**.
[14] A. Akbari niea, A. ghalahvanhi, F. Safidkon, M. B. Rezaei, A. Sharif Ashour Abadi, *Research and construction*., **2004**, 61, 50-32.
[15] H. Rezaei Nejad, R. Omid-Beigi, K. Khademi, *Agricultural Research*, **2000**, 2 (2), 20-13.
[16] L. Hornok, *Acta Horticulturae*., **1986**, 168, 169-176.
[17] A. R. Pirzad, PhD thesis, University of Tabriz (Faculty of Agriculture, **2007**).
[18] J. Lloveras, J. Manent, J. Vioudas, A. Lopez, P. Santiveri, *Agron. J*., **2004**, 96, 1258-1265.

[19] V. E. Tyler, L. Brady, R. Robbers, Pharmacognosy, Pharmacognosy, 9th edn, Lea and Febiger, Philadelphia, **1988**.
[20] S. I. Balbaa, S. Hilal, A. Y. Zaki, Medicinal Plant Constituents, 3rd Edn, Egyptian Dar El-Kotob, Cairo, Egypt, pp 240, **1981**.
[21] E. Güenther, The Essential Oils, Vol 3 and 5, Van Nostrand, Com p. Inc, New York, **1972**.
[22] H. Habibi, D. Mazaheri, N. Majnon Hosseini, M. R. ChaiChi, M. Tabatabai, M. Bigdeli, Ph.D thesis, School of Agronomy and Animal Science, Agriculture and Natural Resources (Tehran University, **2007**).
[23] P. Hormuz Nejad, M. Sc. Thesis, Tarbiat Modarres University (Faculty of Agriculture, **2005**).
[24] W. M. Vital, N. T. Teixeira, R. Shigihara, A. F. M. Dias, *Ecossistema*., **2002**, 27, 69-70.
[25] S. Leithy, T. A. El-Meseiry, E. F. Abdallah, *Journal of Applied Research*., **2006**, 2, 773-779.
[26] F. Moraghabi, H. Pida, H. Hagel Pasand, *Islamic Azad University Science Journal*., **2008**, 18 (1-70), 69-61.
[27] KH. H. Rassam, M. Nadaf, F. Safiidkon, *Journal of Natural Resources and Construction*., **2007**, 75, 132-127.
[28] M. Shareh, M. H. Rashed Mohassel, *pajouhesh sazandegi journal*., **2003**, 75, 127-133.
[29] R. L. Yadava, *Fertilizer- News*., **1984**, 29, 18-25.
[30] M. Malakouti, J. Motasharihzaeh, Role in increasing the quantitative and qualitative improvement of agricultural production, publication Agricultural Education, Karaj, pp 113, **2009**.
[31] L. Hornok, Cultivation and processing of medicinal plants, Akademia Kiado, Budapest, Hungary, pp 200-205, **1992**.
[32] S. Azizi, *J Agr Sci*., **2000**, 3 (123), 88-79.
[33] A. S. Shalaby, A. M. Razin, *J. of Ag. And Cro. Sc*., **1992**, 168, 243-248.
[34] M. Karimi, M. Azizi, Crop growth analysis (translation), Jihad University Press, University of Mashhad, pp 111, **1997**.