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Araştırma Makalesi

Effect of Plant density and Bio–Fertilizer on some morphological traits, seed yield and Yield Components of Sunflower (*Helianthus annus* L.)

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Abstract: This study was carried out at the research farm, faculty of Agriculture Sana'a University during growing seasons of 2012 and 2013. The objectives were to study the effect of two plant densities (5.55, 11.11 plant m^{2-1}) and three levels of Bio-fertilizer fertilization (0, 2, 4 liter ha^{-1}) and on the seeds yield, and it's components of Sunflower plant (Cultivar Euroflor). Experimental results showed variation of plant density effect on examined characters. Head seeds weight and seeds oil content (%) significantly higher with lower plant density (5.55 plant $m^{2})^{-1}$ during first and second seasons, respectively, while higher plant density (11.11 plant m^{2}) positively and significantly influenced on seeds oil % and seeds oil yield during 2012 season. The results showed that the 4 L ha^{-1} Bio-fertilizer level significantly exceeded other Bio-fertilizer concentrations (0 and 2 L ha^{-1}) in its effect on seeds yield, head seeds weight, seeds number/plant, head diameter, seeds oil % and seeds oil yield during first season (2012). During second season (2013), the 4 L ha^{-1} Bio-fertilizer level also surpassed the other levels on each of weight of 1000 seed, leaves number/plant and seeds oil %. Significant interaction between Bio-fertilizer and plant density levels was observed on seeds oil % through first and second seasons. On other studied characters, except plant height, there were interactions only during the first season (2012).

Keywords: Sunflower plant, Bio-fertilizer, plant density, Yemen.

Ayçiçeği (*Helianthus annus* L.) Bitki Yoğunluğu ve Biyo Gübrelemenin Bazı Morfolojik Özellikleri, Tohum Verimi ve Verim Unsurlarına Etkisi

Öz: Bu çalışma, Sana'a Üniversitesi Ziraat Fakültesine ait araştırma çiftliğinde 2012 ve 2013 yıllarında yürütülmüştür. Amaç, iki bitki yoğunluğunun (5.55, 11.11 bitki m^{2-1}) ve üç seviyedeki Bio-Gübre uygulamasının (0, 2, 4 litre ha^{-1}) tohum verimi ve Ayçiçeği bitkisinin Kültivar bileşenleri (Cultivar Euroflor) üzerine etkisini araştırmaktır. Deneme sonuçları, bitki yoğunluğunun incelenen karakterler üzerinde faklı etkide bulunduğunu göstermiştir. Birinci ve ikinci yıllarda baş ağırlığı ve tohumluk yağ içeriği (%) düşük bitki yoğunluğunda (5.55 bitki m^{2-1}) önemli ölçüde daha yüksekken, 2012 sezonunda yüksek bitki yoğunluğu (11.11 bitki m^{2-1}), tohum yağı ve yağ verimi üzerinde pozitif ve belirgin biçimde etkili olmuştur. Sonuçlar, 2012 yılında 4 L ha^{-1} biyo-gübre düzeyinin, diğer biyo-gübre düzeylerine göre (0 ve 2 L ha^{-1}) tohum verimi, baş tohum ağırlığı, tohum sayısı / bitki, baş çapı, tohum yağı (%) ve yağ verimini önemli derecede artırdığını göstermiştir. İkinci yılda (2013), 4 L ha^{-1} biyo-gübre düzeyi, birinci yıla ilave olarak 1000 tane ağırlığı, tohum, yaprak sayısı / bitki ve tohum yağı üzerine diğer dozlardan daha fazla etkili olmuştur. Birinci ve ikinci yıllarda biyo-gübre ve bitki yoğunluğu seviyeleri arasındaki interaksiyonun tohum yağı üzerine önemli etkileşimde bulunduğu gözlenmiştir. Bitki boyu haricinde incelenen diğer parametrelerden yalnızca ilk yılda (2012) etkileşimler gözlenmiştir.

Anahtar Kelimeler: Ayçiçeği bitkisi, Biyo gübre, bitki yoğunluğu, Yemen.

Introduction

Sunflower (*Helianthus annus* L.) is considered the second most important source of vegetable oil in the world (Beard, 1981). It has excellent nutritional properties and its oil is of high unsaturated fatty acids content (Leland, 1996; Seiler, 2007). It is easy cultivated and grown in different climatic conditions and soils (Kaya and Kolsarici, 2011 and Lopez et al., 2011) and grown at a wide range of Latitudes. (F.A.O., 1979; Filipescu and Stoenescu, 1987).

Plant density management is one of the most important considerations that has a large effect on the yield (Li and Xae, 1984; Sarmah et al., 1994 and Alessi and Zimmerman, 1977). The literature on influence of plant density on sunflower productivity has been thoroughly reviewed (Jose et al., 2004; Robinson, 1978). It was mentioned that Sunflower adapts to a range of plant densities. Changes in plant density result in changes in yield components such as number of seeds per head and seed weight.

Sunflower plant densities worked out with a wide range from 30000 plants ha^{-1} (Pala, 1992) to 166600 plants ha^{-1} (Allam et al. 2003) with an average of 98300 plants ha^{-1} or from 6 plants m^{2-1} to 12 plants m^{2-1} (Ali et al., 2012; Ciampitti and Vyn, 2011); Jalilian et al., 2012; Olsen and Weiner, 2007; Rossini et al., 2011) with an average of (9 plants $m^2)^{-1}$. Effect of increasing plant density in sunflower on important plant and yield characters were reported by many researchers such seeds yield (Weiss, 2000), higher yields Beg et al. (2003), maximum grain yield and oil yield (Barros et al., 2004; Wade and Foreman, 1988), plant height (Ali et al., 2012; Ciampitti and Vyn, 2011); Jalilian et al., 2012; Olsen and Weiner, 2007; Rossini et al., 2011), yield and oil content (Sabo and Pepo, 2007), seed and oil yields (Zubriski and Zimmerman, 1974), seed yield, weight of seeds per head, seed size and head and stem diameter (Robinson et al., 1976). With increase of plant population,

above a certain limit, a negative effect on seeds yield through decreased head diameter, number and weight of seeds per head was noticed (Majiri and Arzani 2003). In the other hand, other workers indicated that increased plant density negatively influenced on the sunflower plant and yield characters such as seed yield (Barros et al., 2004), 100 seed weight (Allam et al., 2003), plant height (Sa Xiao and Chen, 2006), yield and oil percentage (Weiss, 1983), number of seeds and diameter of the head (Salehi and Bohrani, 2000).

While some investigators reported reduction in plant density influenced positively on plant and yield characters. Barros et al. (2004) found increase in seed weight, while Zaffaroni and Schneiter (1991) indicated increase of stem diameter, 100 seed weight and seed yield per plant. Head diameter was significantly higher when plant density was reduced (Al-Thabet, 2006; Allam and Galal, 1996; Allam et al., 2003; Salehi and Bohrani, 2000). Oil percentage was gained with sowing at low plant density (Ali et al., 2012; Rao and Reddy, 1985).

Amino acids are involved in the synthesis of other organic compounds, such as protein, amines, alkaloids, vitamins, enzymes and terpenoids (Ibrahim et al., 2010). The role of amino acids, in stimulating cell growth, acts as buffers, provide a source of carbon and energy and protect the cells from ammonia toxicity, with amid formation (Abdel Aziz et al., 2010).

Excessive use of chemical fertilizers in agriculture results in environmental pollution and health problems. Bio-fertilizers are among the sources of plant nutrient and environmentally safe. A part from supplying nutrients for the crop plants, use of Bio-fertilizers reduces the use of chemical fertilizers resulting in environmental conservation. Therefore, Bio-fertilizers are recommended in these conditions as a replacement of chemical fertilizers (Wu et al., 2005). Much attention has been drawn to the application of biological fertilizers in agriculture. In the development and implementation of sustainable agriculture techniques, Bio-fertilization has great importance in alleviating environmental pollution and deterioration of nature (Akbari et al. 2011; JaliIian et al., 2012; Mehran et al., 2011; Soleimanzadeh et al., 2010). Biological fertilizers or Bio-fertilizers promote plant growth through increasing the supply or availability of essential nutrients to the plants (Vessey, 2003). Therefore, many workers studied the effect of Bio-fertilizer application in relation to various concentrations on yield and yield components of sunflower (Abdel-Mawgoud et al., 2011; Vessey, 2003), they found that application of amino acids can stimulate the performance of plant.

There are different methods for application of Bio-fertilizers to plants such as inoculation of seeds before planting or through foliar application. In either of the application method, improvement of plant growth and quality were reported (Tilak, 1992; Rai and Caur, 1998; Abdel Aziz et al., 2010; Amin et al., 2011).

Inoculation of certain microorganism either on pant seeds or directly on soil as Bio-fertilizers for improvement of plant growth and quality was reported by many investigators. Inoculation of sunflower seed with Rhizobacteria proved significant increase in all studied characters (Gorttappeh et al., 2000; Saleh et al., 2004; Zahir et al., 2004), *pseudomonas* (Hernandez et al., 1995; Zahir et al., 2004), *Cerealin* as a N2-fixing bacteria (Keshta and El-Kholy, 1999; Abou Khadrah et al., 2002; Mohamed, 2003) and *Azospirillum* (Fulchieri and Frioni, 1994) and phosphate dissolving bacteria (*Phosphorine*) (Nawar, 1994; Radwan, 1996; Mohamed, 2003; Abou Khadrah et al., 2002).

The yield-contributing characters and quality of plants could be improved by foliar application of putresin and glutamine (Amin et al., 2011), also foliar application of amino acids (tyrosine, thiamine and tryptophan) significantly promoted growth of Thuja orientalis (Abdel Aziz et al., 2010).

Three kinds of amino acid products usually used as liquid formula containing up to nineteen free amino acids in addition to macro and micro elements. Those amino acids proved that they are biologically active and rapidly absorbed activating and regulating plant metabolism.

In Yemen, Massoud (2013) used four levels of Bio-fertilizer (0, 1, 2 and 3 liters ha^{-1}) of 'Ameno Fort' Bio-fertilizer as a foliar application on Sorghum bicolor L. He reported significant increase in number of leaves of /plant, stem diameter, leaf area, leaves stems ratio, number of tillers, dry forage yield, plant height, fresh forage yield, percentage and yield of crude protein with increase of Bio-fertilizer levels. Also Fakirah and Alshabi (2015) used four levels of Bio-fertilizer (0, 2, 4 and 6 liter/ha) at different stages of Zea maize L. plant growth. Their results showed that the highest amount (6 liters/ha) gave significantly higher ear length, weight of cob, number of leaves per plant and grains yield.

Despite the proposed benefits of the application of amino acids on plant growth and safety to environment, in Yemen, still there are not many studies about these kinds of amino acids especially on oil crops. Therefore, the present investigation aimed to evaluate the influence of Bio-fertilizers containing *Amino acids* in various concentrations and plant densities on the yield, yield components, oil concentration and oil yield of sunflower cultivar Euroflor.

Material and Methods

This field experiment was conducted at the Agricultural Experiment Station, Faculty of Agriculture Sana'a University, during growing seasons of 2012 and 2013 to study the effects of different levels Bio-fertilizer (0, 2 and 4 liter ha^{-1}) of Bio-fertilizer and two levels of plant density (D1=5.6 and D2 =11.1 plant m^{2-1}) on the seeds yield and yield components of sunflower plant (Euroflor cultivar). The experimental design used was split-plots design with three replications. The main plots were assigned to the plant densities treatments, whereas the sub-plots were assigned to the Bio–fertilizer. Total experimental units were 18 plots, the main plots separated 2 m apart, similarly the sub–plots to avoiding movement of Bio–fertilizer. Sub-plots consisted of five rows, 3.0 m long and 60 cm apart with 30 cm distance between hills (total area of 9 m^2). The seeds were sown on the 1st of Jun of the both growing seasons (2012, and 2013). After 20 days from sowing, the plants were thinned according to required plant densities (one plant and two plants/ hill). Weeds were controlled manually throughout the experiment. Recent soil analysis data of the experimental site was collected from Laboratory of Soil and Water Department. Soil analysis data are given in Tables (1 & 2).

Table 1. Physical properties of soil of experimental site

Soil Depth (cm)	EC (dS/m)	Clay (%)	Silt (%)	Sand (%)	Soil Texture
0 – 30	0.4	15.50	25.80	58.70	Sandy Silt

Table 2. Chemical properties of soil of experimental

Soil Depth (cm)	pH	Organic Matter (%)	Total Nitrogen (%)	Phosphor (mg/kg)	Potassium (mg/kg)
0 -30	8.30	0.95	0.09	5.80	150.13

Bio-fertilizer treatments

A set of Bio-fertilizers is used. It consisted of three commercial products namely Humiforte, Fosnutren and Kadostim. Foliar application method was adopted through four stages to each sub–plot. Stages were seedling stage, vegetative stage, flowering stage, and seed formation stage. Three concentrations (0, 2 and 4 L ha^{-1}) of each Bio-fertilizer were applied as recommended amount (liter). Fosnutren was applied at seedling stage 20 day after sowing, Humiforte was applied at vegetative stage 40 days after sowing, Fosnutren also was applied at flowering stage 65 days after sowing (50% flowering), and Kadostim was applied at seed formation stage, 85 days after sowing. Concentrations of Bio–fertilizer (2 and 4 liters) were prepared by dissolving each concern Bio-fertilizer in irrigation water at the rate of 1 ml L 1 liter Bio-fertilizer per 1000 liter of irrigation water. The source of the three kinds of Bio-fertilizers was from Inagrosa Industrials Agrobiologic as S.A -Spain (Table 3).

Table 3. Chemical composition of Bio-fertilizers used to study their effect on some morphological traits, seed yield and yield components of sunflower (*Helianthus annus* L.)

Names of Bio–fertilizer	**Active ingredients &Nutrients**	**Content of Amino acids**
Fosnutren	Nutrients	Phosphorus 6.10 %; Iron 0.12 %; Manganese 0.06 % and Copper 0.08 %
	Amino acids	Hydroxy proline 11.4 %; Tyrosine 0.9 %; Aspartic acid 5.7%; Phenylalanine 2.1 %; Threonine 1.8 %; Lysine 3.3 %; Serine 3.7% and Histidine 0.9 %.
	Density	1.07
	pH	4.7
Humiforte	Nutrients	Total Nitrogen 6 %; Phosphorus ($P_2 O_5$) 3%; Potassium (K2o) 5%; Zinc 0.09 %; Iron 0.12 %; Copper 0.08 % and Manganese 0.06 %.
	Amino acids	Proline 13.4%; Valine 2.2 %; Glutamic acid 9.8 %; Leucine 2.8 %; Glycine 20.3 %; Alanine 8.5 and Arginine 11.7 %.
	Density	1.10
	pH	5.8
Kadostim	Nutrients	Potassium ($K_2 0$) 6%; Zinc 0.09 %; Iron 0.12 %; Copper 0.08 % and Manganese 0.09 %.
	Amino acids	Methionine 0.6% and Isoleucine 1.3 %.
	Density	1.11
	pH	7.3

At the end of the experiment in each season, ten plants were selected randomly from each sub-plot for data collection of the following traits:

1- Head seed weight (g); 2– Seeds number per plant; 3- Leaves number/plant 4- plant height (cm).

The rest of plants of middle rows in each sub-plot were harvested with a total area 5.4 m^2 of each sub-plot. Harvested heads were oven dried at 60 °C for 24 hours and seeds were thrashed.

The following characters were studied: 1- Seeds yield kg ha^{-1}; 2- 1000 seed weight (g); 3- Seed oil content (%).

Oil content of each seed sample was determined according to AOCS (1980). Data of the studied characters were analyzed by SAS statistical program (1992).

Results and Discussion

Seeds yield (kg ha^{-1}):

The results (Table 4) revealed that, there was no significant effect of plant densities on seed yield through the two seasons of the experiment. Increasing plant densities from 5.55 to 11.11 plants m^{2-1} had no significant effect on seed yield. This result was supported by Majid and Schneiter (1987) and Pala (1992). Their studies indicated that the seeds yield were not affected by increasing plant densities explaining that the optimum plant density for seeds yield depends on the cultivar and environment. On the other hand results were previously reported indicating that increase plant population significantly increase seed yield (Zubriski and Zimmerman, 1974, Beg et al., 2003, Robinson et al., 1976, Barros et al., 2004; Sabo and Pepo, 2007; Weiss, 2000 and Wade and Foreman, 1988). Whereas other investigators reported that increasing of plant population decreased seed yield (Weiss, 1983 and Barros, 2004).

Increasing Bio–fertilizer concentrations from 0 - to 4 L ha^{-1} was clear on seeds yield in the first season 2012 (Table 4). There was significant difference between treatments where the application of 4 L ha^{-1} Bio–fertilizer differ significantly on control gave the higher value (1579.6 kg ha^{-1}). This might be due to the need of increasing Bio–fertilizer concentrate with increase of plant growth stage. Resulting in the increase in seed yield, which might be due to improve in translocation of assimilates, Similar results obtained by (Abdel-Mawgoud et al., 2011). Vessey (2003) also found components of yield of sunflower related directly with varying concentrations of Bio-fertilizer application concentrations and the application of amino acids can stimulate the performance of plant. Similarly, increase of Bio-fertilizer concentration in maize encouraged seed yield (Fakirah and Alshabi, 2015).

The great values of seed yield were produced from sunflower plants that received Bio-fertilizer, containing 6.10% phosphorus and 6% potassium in addition to other elements and amino acids during flowering and seeds formation stages. Similar results were obtained by Mohamed (2003); Keshta and El- Kholy (1999); Gorttappeh et al. (2000); Abou Khadrah et al. (2002); Saleh et al., (2004); Amin et al. (2011) and Kesba (2003) that they reported all concentration levels of amino acid tested, significantly increasing plants growth of sunflower and grapes, whereas increasing mineral nitrogen rate with Bio-fertilizers and Farm Yard Manure (FYM) significantly increased, seed yield, Inoculation of sunflower

seed with Rhizobacteria plus application of Nitrogen caused significant increase of all studied characters. Also, the inoculation of sunflower seed with N_2-fixing bacteria (*Cerealin*) plus phosphate dissolving bacteria (*Phosphorine*) significantly enhanced seeds yield. On the other hand, the applications of inorganic nitrogen and Bio-fertilizers as a source of N_2 fixing bacteria for sunflower increased seed yield/fed. Whereas increasing in application rates of nitrogen fertilizer and manure caused increased seeds yield. Same results was obtained in maize, by Zahir et al. (2000) who found increased grain yield by inoculated seeds with *Azotobacter* and *Pseudomonas*.

Table 4. Effect of plant Density and Bio–fertilizer on yield, yield components and oil yield of sunflower, season 2012.

Treatments		Seeds yield (kg ha^{-1})	Head seeds weight (g)	Seeds number/plant	Seeds weight /1000 (g)	Head diameter (cm)	Leaves number/ plant	Plant height (cm)	Seeds oil content (%)	Seeds oil yield (kg /ha)
Plant Density (Plants/ m^2)	5.55	1157.5^a	24.37^a	439.00^a	49.98^a	9.21^a	32.34^a	158.72^a	39.96^b	464.05^b
	11.11	1644.2^a	17.64^b	385.54^a	50.53^a	8.84^a	32.44^a	166.67^a	44.16^a	729.48^a
L. S.D.(0.05)		516.14	6.50	57.97	0.89	1.10	6.17	40.62	0.00	218.88
Bio–fertilizers concentrates (liter/ ha)	0	1244.7^b	17.00^b	344.40^b	46.12^a	8.22^b	31.53^a	156.90^a	39.51^c	492.50^b
	2	1378.2^{ab}	22.60^{ab}	432.98^a	50.52^a	9.06^{ab}	32.93^a	164.50^a	42.39^b	592.66^{ab}
	4	1579.6^a	23.43^a	459.44^a	54.13^a	9.79^a	32.71^a	166.69^a	44.28^a	705.14^a
L.S.D. (0.05)		278.62	6.03	72.40	11.64	1.07	1.67	14.28	0.00	121.63

The interaction between Bio-fertilizer and plant densities had significant effect on seed yield (kg ha^{-1}), the treatment of application of 4 L ha^{-1} on cultivated plants at 11.11 m^2 density, achieved the highest seed yield (1765.8 kg ha^{-1}) Figure (1-A).

Head seeds weight (g):

The results indicated negative correlation between plant density and head seed weight in the same season. Head Seed weight in the lower plant density (5.55 plant m$^{2-1}$) showed the highest value (24.373 g) while the highest plant density gave the lowest (17.644 g) head seed weight (Table 4). This result was supported by Majiri and Arzani (2003); Al–Thabet (2006) that indicated increase plant density resulted in decrease of head seeds weight of sunflower plant. This may be due to decrease in head diameter that may be due to reduction in normal seed development either to lack of some flower pollination and or competition on food sources, moisture and sunlight. On the other hand, our findings are not in agreement with results reported by Allam et al. (2003); Salehi and Bohrani (2000), Barros et al. (2004), Robinson (1976); Zaffaroni and Schneiter (1991) and Pala (1992) whose results revealed that increase head seeds weight was associated with increase plant density.

Higher head seeds weight character was responded positively to increase in Bio-fertilizer concentration in the first season in gradual manor between 17.002 g for the control and 23.427 g when 4 liter Bio-fertilizer per hectare (Table 4).

The highest value of head seed weight was produced from sunflower plants received Bio-fertilizer that contain the 6.10% phosphorus, 6% potassium plus other elements and amino acids during flowering and seeds formation stages. The increase in head seed weight might be due to improve in translocation of assimilates and the role of Bio-fertilizer in relation with different concentrations on yield of sunflower and its components yield. Our results are in agreement with those of Abdel-Mawgoud et al. (2011); Amin et al. (2011). Similarly, Nawar (1994) and Radwan (1996) who reported that inoculation of sunflower seed with phosphate dissolving bacteria (phosphorine) significantly increased weight of seeds/head.

The interaction between Bio-fertilizer and plant densities had significant effect on head seed weight. The using 4 L ha^{-1} on plant cultivated at 5.55 plant m^{2-1} resulted in the highest head seed weight (28.000 g) Figure (1-B).

Seeds number/plant:

The character of seed number per plant was not significantly influenced by different pant density levels in both seasons (Tables 4&5).

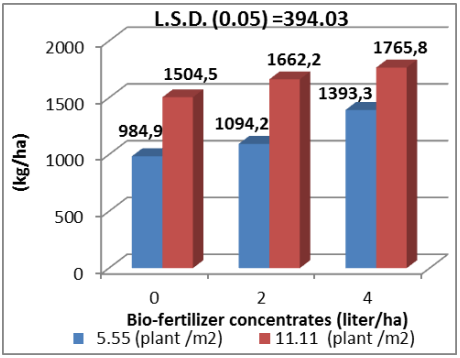
Similarly, Majid and Schneiter (1987) identified that increased plant population in a unit area had no significant effect on seed number per plant. However, others studies (Salehi and Bohrani, 2000; Majiri and Arzani, 2003) indicated that number of seed per plant decreased with increasing plant density, explaining that the cause might by due to reduction of head diameter. In other studies (Al-Thabet, 2006); Allam and Galal, 1996 and Salehi and Bohrani, 2000) conclusion head diameter was significantly increased as the space between plants was increased indicating that progressive increase in number of grains per head associated with increase in plant spacing.

The results in Table (4) showed significant differences between Bio–fertilizer treatments on seed number per plant in the first season (2012). Increase of Bio–fertilizer concentrations concentration from (0-4 L ha^{-1}) had major effect on seed number per plant. The higher level treatment gave the highest value (459.44 seeds/plant, Table 5). Increase in seeds number per plant might be due to the importance of increase head diameter with increase in the Bio-fertilizer concentrations from 0-4 L ha^{-1}).

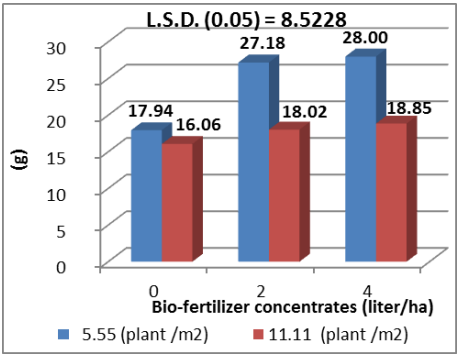
Table 5. Effect of plant Density and Bio–fertilizer on yield, yield components and oil yield of sunflower, season 2013.

Treatments		Seeds yield (kg ha^{-1})	Head seeds weight (g)	Seeds Number /plant	Seeds weight /1000 (g)	Head diameter (cm)	Leaves number /plant	Plant height (cm)	Seeds oil content (%)	Seeds oil yield (kg ha^{-1})
Plant Density (Plants/ m^2)	5.55	1279.2[a]	13.10[a]	363.90[a]	40.17[a]	8.25[a]	30.74[a]	135.92[a]	40.20[a]	515.50[a]
	11.11	2065.0[a]	12.23[a]	320.70[a]	37.86[a]	7.43[a]	30.36[a]	131.02[a]	39.05[b]	815.2[a]
L.S.D.(0.05)		1493.5	15.30	440.47	9.99	3.36	4.64	55.97	0.08	605.53
Bio–fertilizers concentrates (liter/ ha)	0	1243.8[a]	9.53[a]	297.27[a]	33.80[b]	6. 70[a]	26.78[b]	125.03[a]	37.78[c]	466.10 [a]
	2	1814.0[a]	13.02[a]	400.98[a]	38.58[ab]	7.76[a]	31.70[a]	133.27[a]	39.87[b]	724.40[a]
	4	1958.4[a]	15.44[a]	328.74[a]	44.67[a]	9.07[a]	33.17[a]	142.12[a]	41.22[a]	805.40[a]
L.S.D. (0.05)		1084.3	8.3202	190.88	8.4272	2.7165	4.5493	20.318	0.049	439.38

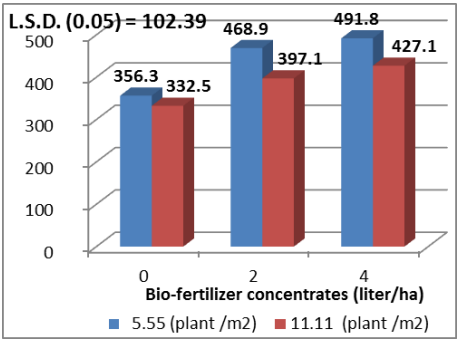
The interaction between Bio-fertilizer and plant densities had significant effect on seeds number/plant. The highest head seed number (491.8 seeds) was achieved when plants were cultivated in lower density and received 4 liters Bio-fertilizers/ha (Figure, 1-C).



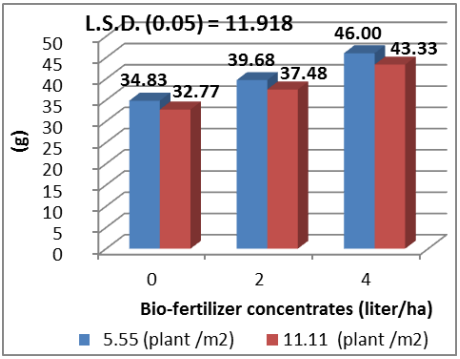
A: Seeds yield



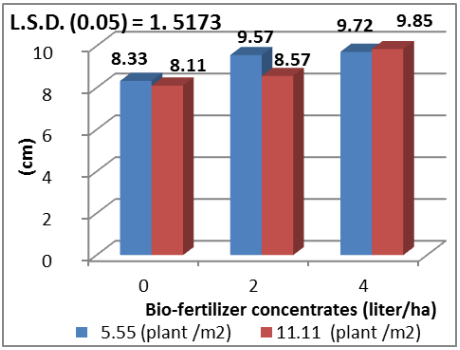
B: Head seeds weights



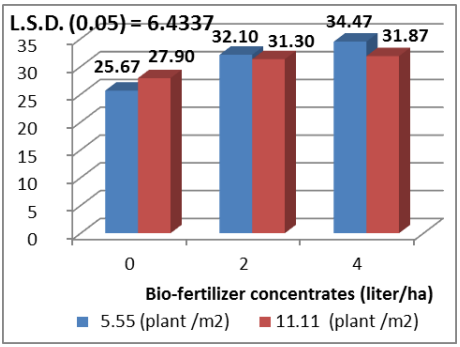
C: Seeds number/plant



D: 1000 Seeds weight

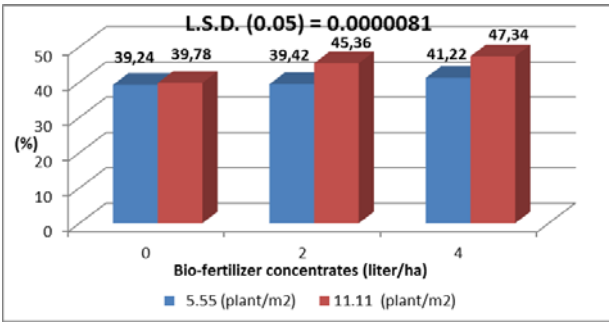


E: Head diameter

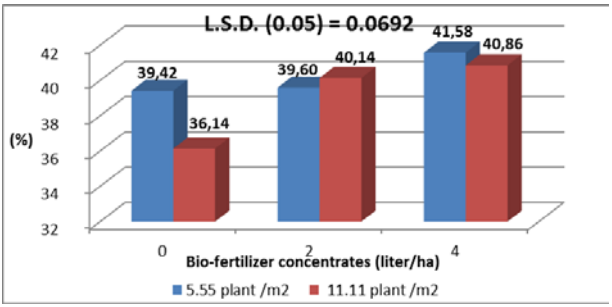


F: Leaf number/plant

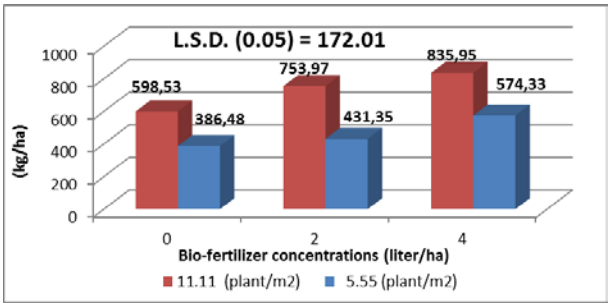
Figure 1. Interaction between Bio-fertilizer and plant densities on seeds yield (A), head seeds weights (B), seeds number/plant (C), 1000 seeds weight (D), head diameter (E) and leaf number/plant (F), during first season (2012).



A: Seeds oil content during first season



B: seeds oil content during second season



C: Seeds oil yield during first season

Figure 2. Interaction between Bio-fertilizer and plant densities on seeds oil content during first season (A) 2012 and (B) second season (2013) and seeds oil yield during first season (C).

Weight of 1000 seed (g):

The results indicated no significant differences between density levels (Tables 4 & 5). Our results are not in agreement with those of Majid and Schneiter (1987) where they found significant decrease in 1000 seed weight with increase plant density. Similarly, Alam et al., (2003) reported the 1000 seed weight and seed size were decreased by increasing plant population. On the other hand, Zaffaroni and Sohneiter (1991) and Barros et al., (2004)

indicated that decreased plant density increased 1000 seed weight. This might be due to less competition for water and nutrient leading to reduced seed development.

Significant variations among the Bio–fertilizer treatments were clear in the second season (Table 5). Increased the Bio–fertilizer concentration level from 0-4 liter ha^{-1} significantly increased 1000 seed weight (g). Higher concentration gave the higher value (44.667 g), while the lower weight (33.800 g) was given by the control. This significant difference might be due to presence of 6% potassium plus other elements and amino acids during seeds formation stages in treated plants with 2 and 4 L ha^{-1} Bio-fertilizers that was absent in the control treatment. This unblemished the role of potassium in metabolic processes of plants particularly in the metabolism of carbohydrates. Similar results obtained by Abdel-Mawgoud et al. (2011) who interpreted increased in the 1000 seed weight with increase of bio-fertilizer level was due to improvement in translocation of assimilates and the role overall of Bio-fertilizers in on yield of sunflower and its components.

Increasing mineral nitrogen rates with bio and FYM significantly increased, 100-seed weight (Gorttappeh et al., 2000; Abou Khadrah et al., 2002; Mohamed, 2003), likewise results obtained by Keshta and Kholy (1999) indicated the application of inorganic nitrogen and Bio-fertilizers as a source of N_2 fixing bacteria for sunflower increased 100 seed weight.

The interaction between Bio-fertilizer and plant densities had significant effect on 1000 seed weight. The treatment Application of 4 liter Bio- fertilizer ha^{-1} on lower density sunflower plants achieved highest weight of 1000 seed (Figure 1-D).

Head diameter (cm):

The results (Tables 4 & 5) indicated that there were no significant differences between the means of head diameter among density levels (5.11 and 11.11 plant/m^2 both growing seasons. These results, in general, are not in agreement with those obtained by Salehi and Bohrani (2000); Majiri and Arzani (2003) who reported that there was decrease in head diameter by increasing plant density. Sa Xiao, (2006) found the increased of plant densities caused increased head diameter, whereas others (Allam and Galal, 1996; Salehi and Bohrani, 2000; Al-Thabet, 2006) reported that decreased of plant density enhanced increase in head diameter.

Mean results (Table 4) indicated that the Bio-fertilizers treatments showed significant differences on head diameter, in the first season 2012. These differences were correlated with the increased of Bio–fertilizer concentrations. The higher concentration (4 L ha^{-1}) gave the highest value (9.7867 cm), followed by the median (2 L ha^{-1}) which gave (9.0667 cm). Whereas the control (0 L ha^{-1}) gave the lowest (8.2200 cm) value (Table 4). The results showed, that head diameter increased by the increased of Bio–fertilizer concentrations from (0 L ha^{-1}) to (4 L ha^{-1}). This might be due to the need to increase Bio–fertilizer concentrate in different stages of plant growth. Similar results obtained by Abdel-Mawgoud et al., (2011) indicated the importance of Bio-fertilizer application for various yield components of sunflower. In addition, the application of amino acids overall stimulate the performance of plant. The increased in head diameter might be interpreted by improve in translocation of assimilates. The great values of head diameter produced from sunflower plants fertilized with the Bio-fertilizer which contains 6% nitrogen incorporation with other elements plus

amino acid during vegetative stage. Similar results observed by Abou khadrah et al. (2002). Mohamed (2003) who reported increasing mineral nitrogen rate with bio and FYM significantly increased dry matter accumulation, head diameter in sunflower plants. Others; Nawar, (1994) and Radwan (1996) reported that inoculation of sunflower seed with phosphate dissolving bacteria (*phosphorine*) significantly increased head diameter. On the other hand, results obtained by Keshta and El-Kholy (1999) indicated increased, head diameter by application of inorganic nitrogen and Bio-fertilizers as a source of N_2 fixing bacteria.

The interaction between Bio-fertilizer and plant densities had significant effect on head diameter. The application of Bio-fertilizer at the rate of 4 L ha^{-1} on sunflower plants at 11.11 plant ha^{-1} resulted in highest (9.8533 cm) head diameter Figure 1-E).

Leaves number/plant:

Increasing of plant density, from 5.55-11.11 plants m^{2-1}, did not significantly influence on leaf number per plant in both seasons (Tables 4 & 5). Similar result obtained by Amjed et al. (2011) reported the effect of plant spacing on the number of leaves per plant was not significant. These results are also supported by Bakht et al. (2006), who observed significant differences for number of leaves among different hybrids.

Differences due to Bio–fertilizers treatments were significant in 2013 season (Table 5). The third treatment (4 L ha^{-1}) gave the highest value (33.167 leave) which was on par with median rate of bio-fertilizer (2 L ha^{-1}), which gave the value of 31.700 leave/plant. The increased in this trait might be due to positive reaction of plants received Bio-fertilizer with nitrogen (6%) plus other elements and amino acids at vegetative stage. This is also emphasis on the role of nitrogen fertilizer in biological activity and improvement of plant metabolism. Similar results, in Sunflower, were obtained by Abou Khadrah et al. (2002); Mohamed (2003), and Vessey (2003) who reported that biological nitrogen fertilizer and Bio-fertilizer promote plant growth through increasing availability of essential nutrients to the plants that improve plant metabolism reflected through overall plant growth among which number of leaves per plant is one. It was also reported in Maize (Fakirah and Alshabi, 2015) and Sorghum (Massoud, 2013).

The interaction between Bio-fertilizer and plant densities had significant effect on leaves number/plant, the treatment of application of Bio-fertilizer at the rate of 4 L ha^{-1} on sunflower plants at 55.55 plant/ha achieved the highest number of leaves per plant (Figure 1-F).

Plant height (cm):

Increasing plant density from 5.55 to 11.11 plants m^{2-1} had no significant effect on plant height (Tables 4 & 5). These results are not in agreement with those obtained by Sa xiao et al. (2006) who reported that plant height decreased with increasing plant population in the field. While others such as Ciampitti and Vyn (2011); Jalilian et al. (2012); Olsen and Weiner (2007) and Rossini et al. (2011) reported increase of plant density significantly increase plant height.

Our results are not in line with the findings of other researchers, (Ciampitti and Vyn, 2011; Jalilian et al., 2012; Olsen and Weiner, 2007; Rossini et al., 2011) who reported inoculation of plants with bio-fertilizer increased plant height than non-inoculated plants.

Seeds oil content (%):

The plant densities, significantly effect on seed oil content. Results (Table 4) showed that the increased plant densities resulted in increased seed oil content in the first season 2012, whereas in the second season (2013) the decreased plant densities caused increased seed oil content (Table 5). In the first season, higher value of 44.160% was obtained with higher plant density, while, in the second season, higher value of 40.200% was obtained with low plant density. Fluctuation of seed oil content in different seasons seems to be a common. Findings of many researchers (Sabo and Pepo, 2007; Weiss, 1983; Alessi et al., 1977; Jones, 1978; Prunty, 1983; Rao and Reddy, 1985; Sabo and Pepo, 2007; Namvar et al., 2012) in increasing and decreasing oil content showed non-constant results with varying plant densities. This might be due to that the seed from the higher plant density may have had a thinner pericarp, which resulted in slightly higher oil content. Also, oil percentage of sunflower seeds depends on ratio of both percentages of hull and oil content in kernel.

The mean of seed oil content differed significantly between all levels of bio-fertilizer, including the control, in both seasons (Tables 4 & 5). It appears from the results of both seasons a constant increase of seed oil content with increase of Bio-fertilizer concentration. The range of oil content was between 39.51 – 44.28% in the first season and 37.78 – 41.22% in the second season.

The increase in seed oil content might be due to increase of phosphorus element as a result of increase of Bio–fertilizer level. Mohamed (2003) and Abou Khadrah et al., (2002) reported that data revealed that the inoculation of sunflower seed with combined of the (N_2-fixing) bacteria (*Cerealin*) and phosphate dissolving bacteria (*Phosphorine*), the two bio-fertilizers significantly enhanced seed oil content. Similar results were previously reported by Mohamed (2003) and Abou Khadrah et al., (2002) who reported that the inoculation of sunflower seed with combined of the (N_2-fixing) bacteria (*Cerealin*) and phosphate dissolving bacteria (*Phosphorine*), significantly enhanced seed oil content. Application of Bio-fertilizers, generally, improved plant growth, particularly when including amino acids. Similar results were previously reported, (Abdel-Mawgoud et al., 2011; Amin et al., 2011; Kesba, 2003) indicated improvement of yield characters and overall quality of plant growth with application amino acid concentrations on sunflower and grapes. It could be concluded that the increased oil content might be due to receiving of sunflower plants to Bio–fertilizer, which contain 6.10% phosphorus plus other elements and amino acids during seedling and flowering stages. The interaction between Bio-fertilizer and plant densities had significant effect on seeds oil content (%), the treatment of applying 4 liter Bio-fertilizer ha^{-1} on sunflower plants cultivated on higher density (11.11 plants m^{2-1} achieved the highest seed oil content at 47.34% (Figure 2-A). While in the second season, the interaction achieved highest seed oil content (41.58%) was when applied Bio-fertilizer at higher rate (4 L ha^{-1}) on plants cultivated at lower density at 5.55 plants m^{2-1} (Figure 2-B).

Seeds oil yield (kg/ha):

The results (Table 4) indicated significant influence of plant density on oil seed yield. Seeds from plants at higher plant density (11.11 plant m^{2-1}) gave the higher seed oil yield (729.48 kg ha^{-1}), while seed oil yield from plant cultivated at low plant density (5.55 plants/m^{2-1}) reduced by almost 37% compare to seed oil yield from the higher density cultivated plants.

There was significant difference between Bio–fertilizer received plants and control (Table 4) higher rate of Bio-fertilizer (4 L ha^{-1}) gave higher seed oil yield (705.14 kg ha) that exceeded significantly the production of oil (492.50 kg ha^{-1}) form control treatment (0 L ha^{-1}) by approximately 70%. Since values of seed oil yield is a calculation of seed yield multiplied by seed oil content. So the interpretation of the increased seed oil yield with increase Bio-fertilizer levels might be due to the role of bio-fertilizers promote plant growth through increasing the supply or avail of essential nutrients to the plants in increasing seed oil yield as increase seed yield and seed oil content . This agreed with what explained by Vessey (2003). Overall, Phosphorus played an essential role in photosynthesis, stimulating early root growth and promoting early plant vigor (Longstreth and Noble, 1980; Verberic et al., 2002).

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