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# Effect of Biological and chemical fertilizers on medicinal pumpkin features

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Cropping systems in farmland areas of Iran are characterized by consumption of chemical fertilizers (C) leading to serious erosion and fertility decline. Information regarding the influence of bio, organic fertilizers (O) and C on pumpkin traits is not available. Therefore, a field experiment was conducted at research farm of Kurdistan university in 2009. Fourteen compositions of bio, O and C were done as treatments. Results indicated that the maximum seed yield ( $118.5 \text{ g m}^{-2}$ ), oil yield ( $43.31 \text{ g m}^{-2}$ ) and fruit yield ( $6056.2 \text{ g m}^{-1}$ ) were obtained with treatment that pumpkin seeds inoculated with free-living nitrogen fixing bacteria (NO) and phosphate solubilizing bacteria (PO) + 50% organic fertilizer. Oil percentage responded to fertilizer treatments and the maximum oil percentage (48.15%) was obtained at treatment that pumpkin seeds inoculated with PSB + 50% chemical fertilizer. The fertilizer treatments had no significant effects on the 1000 seeds weight and fruit number per plant. It is concluded that application of biofertilizers with 50% chemical and organic fertilizers (CO), reduced the need for C and produced higher oil, seed and fruit yield.

**Key words:** Biofertilizer, compost, farmyard manure, oil, pumpkin.

## INTRODUCTION

Medicinal pumpkin (*Cucurbita pepo* subsp. *Pepo* convar. *pepo* var. *Styriaca*) is an important species and widely used in traditional and industrial pharmacology (Ghaderi et al., 2008). This plant has been formed by a spontaneous mutation mutant of standard pumpkin (*C. pepo* L.). The result of this mutation is a single recessive

gene that produce a very thin outer hull (naked or hull-less seeds), which highly facilitates the production of this regional specialty oil (Košťálová et al., 2009; Fruhwirth and Hetmetter, 2007). The length of its sprawling prickly stem is between 300-500 cm. The fruits are green to orange and the root systems are fibrous (Gholipouri and Nazarnejad, 2007). Flowers are bright-yellow, leaves are large and green and seeds are dark green and coverless (Nikkhah et al., 2009). The nutritional value of pumpkin seeds is based on high protein content (25-51%) and high percentage of oil that ranging from 40-60%, up to 60.8% (Abdel-Rahman, 2006). The oil contains the fatty acids, 25-35.9% oleic and 40.4-55.6% linoleic acid, stearic and palmitic acids typically ranged from 5.2-7.5% and 6.2-12.4%, respectively (Applequist et al., 2006). Pumpkin oil is unsuitable for cooking and frying because of its dark green color and high content of unsaturated fatty acids (up to 78 %). Heating decreases the content of linoleic (C 18:2) acid while increasing the stearic (C 18:0) acid content (Krička et al., 2005). The grains of pumpkin contain medicinal raw materials that are used for producing pharmaceutical products such as peponen, pepostrin and gronfing to overcome prostatic hypertrophy

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**Abbreviations:** PSB, Phosphate solubilizing bacteria; NFB, nitrogen fixing bacteria; GLM, general linear model; ANOVA, analysis of variance; LSD, least significant difference; SAS, statistical analysis system; S, control; N, inoculated pumpkin seeds with NFB; P, inoculated pumpkin seeds with PSB; C, chemical fertilizer; O, organic fertilizer; NP, NFB + PSB; NO, NFB+ 50% organic fertilizer; NC, NFB+ 50% chemical fertilizer; PO, PSB + 50% organic fertilizer; PC, PSM + 50% chemical fertilizer; CO, 50% organic fertilizer + 50% chemical fertilizer; NPO, NFB+ PSM + 50% organic fertilizer; NPC, NFB+ PSB + 50% chemical fertilizer; NPCO, NFB+ PSB + 50% organic fertilizer+ 50% chemical fertilizer;

and urinarytract irritation (Sedghi et al., 2008). These seeds showed good results in the therapy of minor disorders of the prostate gland and the urinary bladder (Peričin et al., 2008).

For optimum plant growth, nutrients must be available in sufficient and balanced quantities (Chen, 2006). Farming regions that emphasize heavy chemical application are led to adverse environmental, agricultural and health consequences (Shehata and El-khawas, 2003). One of the possible options to reduce the use of chemical fertilizers could be use of bio and organic fertilizers. Biofertilizers are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi et al., 2008). Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid et al., 2007). To increase the availability of phosphorus and nitrogen for plant, large amounts of fertilizers are used on a regular basis soon after application of a large proportion of phosphorus fertilizer is rapidly immobilized and becomes unavailable to plants (Xiao et al., 2008) and also, 25% of the applied nitrogen fertilizer is lost from the soil plant system through leaching, volatilization and denitrification (Saikia and Jain, 2007). Symbiotic nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar et al., 2009). The fixed phosphorus in the soil can be solubilized by phosphate solubilizing bacteria (PSB), which have the capacity to convert inorganic unavailable phosphorus form to soluble forms  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  through the process of organic acid production, chelation and ion exchange reactions and make them available to plants. Therefore, the use of PSB in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble in the fertilizers and soils to which they are applied (Chang and Yang, 2009; Banerjee et al., 2010).

Biological nitrogen fixation is one way of converting elemental nitrogen into plant usable form (Gothwal et al., 2007). Nitrogen-fixing bacteria (NFB) that function transform inert atmospheric  $\text{N}_2$  to organic compounds (Bakulin et al., 2007). The ability of these bacteria to contribute to yields in crops is only partly a result of biological  $\text{N}_2$ -fixation. The Mechanisms involved have a significant plant-growth promotion potential. In these relationships the bacteria receive non-specific photosynthetic carbon from the plant and, in turn, provide the plant with fixed nitrogen, hormones, signal molecules, vitamins, iron, etc (Kavadia et al., 2007; Mikhailouskaya

and Bogdevitch, 2009). Previous studies showed that the combination of biofertilizers with organic or chemical fertilizers further enhanced the biomass and grain yield of crops (Azzan et al., 2009; Yasari et al., 2008; Anjum et al., 2007).

Soils of arid and semi-arid regions have low organic carbon content and need organic amendments to improve their properties and consequently their productivity and natural fertility. Addition of organic matter, from different sources, improved physical and chemical properties of soil and consequently affects the growth and development of plant roots and shoots (El-ashry et al., 2008).

Although, information on the effect of animal manure and the other organic fertilizers on crop yield are available, farmers in the Iran rely on chemical fertilizers to maintain crop yield, and pay little attention to maintaining soil organic matter in soils in Iran (Shirani et al., 2002). Some manure- bound nutrients are gradually released from the organic component of the manure. The release rates of these organic nutrients can conceivably be affected by the chemical nature of the manure and SOM that forms from the manure (Mao et al., 2008). The use of animal waste in maintaining soil organic matter is a popular practice in all parts of the world. It is not only a safe and effective way of recovery for lost plant nutrients like nitrogen and phosphorus but also improves the physical and chemical attributes of the soil (Azeez et al., 2010).

Municipal solid waste can be composted to reduce the volume of waste and disease-causing organisms and to convert it in an organic-rich, soil-like product, through aerobic or anaerobic fermentation (Hussain et al., 2007). The addition of municipal solid waste compost to agricultural soils has beneficial effects on crop development and yields by improving soil physical and biological properties (Gharib et al., 2008). Positives effects of organic fertilizers on growth of pumpkin were reported in several studies (Warman et al., 2009; Awondum, 2007).

The objective of this study was to determine the yield and oil content of medicinal pumpkin plant as influenced by the application of biofertilizers, organic and mineral fertilizers. The goal was to minimize inorganic fertilize usage.

## MATERIALS AND METHODS

### Field experiments

The field experiment was conducted on sandy loam soil at the Research Farm of University of Kurdistan in Sanandaj, Iran. The area is located at latitude of  $35^{\circ}15' \text{N}$  and longitude of  $47^{\circ}1' \text{E}$  at an altitude of 1300 m above the sea level. Chemical properties of the soil used in this study are presented in Table 1. The experiment was carried out in a randomized complete block design with 3 replications and 14 treatments including: control (S), inoculated pumpkin seeds with NFB (N), inoculated pumpkin seeds with PSB

**Table 1.** Soil characteristics in research field.

| Soil texture | Clay (%) | Sand (%) | Silt (%) | pH  | EC (dSm <sup>-1</sup> ) | OC (%) | N (%) | P (ppm) | K (ppm) | Zn (ppm) | Fe (ppm) | Cu (ppm) |
|--------------|----------|----------|----------|-----|-------------------------|--------|-------|---------|---------|----------|----------|----------|
| Sandy loam   | 16%      | 58%      | 26%      | 7.9 | 0.61                    | 1.46   | 0.21  | 5.14    | 82.95   | 5.56     | 12.19    | 2.3      |

**Table 2.** The results of variance analysis of fertilizer treatments on yield and yield components of pumpkin.

| SOV       | df | Mean square         |                          |                    |                     |                            |                          |                       |
|-----------|----|---------------------|--------------------------|--------------------|---------------------|----------------------------|--------------------------|-----------------------|
|           |    | 1000 seed Weight    | Number of seed per fruit | Fruit weight       | Fruit diameter      | Number of fruits per plant | Fruit yield              | Seed yield            |
| Block     | 2  | 329.89              | 1089.45                  | 0.45               | 17.92               | 0.0055                     | 283881.16                | 79.61                 |
| Treatment | 13 | 272.3 <sup>ns</sup> | 16015.26 <sup>**</sup>   | 0.95 <sup>**</sup> | 154.1 <sup>**</sup> | 0.071 <sup>ns</sup>        | 5112843.92 <sup>**</sup> | 1382.56 <sup>**</sup> |
| Error     | 26 | 285.48              | 2485.94                  | 0.15               | 33.2                | 0.04                       | 864353.01                | 225.35                |
| CV        |    | 12.29               | 20.37                    | 20.39              | 11.17               | 16.49                      | 21.01                    | 20.48                 |

ns, Not significant; \*\*, significant in 1% probability level; \*, significant in 5% probability level.

(P), chemical fertilizer (C) (urea+ mono super phosphate), organic fertilizer (O) (animal manure+ compost), NFB + PSB (NP), NFB+ 50% organic fertilizer (NO), NFB+ 50% chemical fertilizer (NC), PSB + 50% organic fertilizer (PO), PSM + 50% chemical fertilizer (PC), 50% organic fertilizer+ 50% chemical fertilizer (CO), NFB+ PSM + 50% organic fertilizer (NPO), NFB+ PSB + 50% chemical fertilizer (NPC), NFB+ PSB + 50% organic fertilizer+ 50% chemical fertilizer (NPCO). After the land preparation such as, plowing, disking and ridging, medicinal pumpkin was sowed in five- row plot with a plant to plant distance of 50 cm and row to row distance of 1 m, Three seeds per hole were placed at 3 cm planting depth. The plants were thinned to one at the three to four leaf stages. The P (150 kg ha<sup>-1</sup>) and organic fertilizers (10 t ha<sup>-1</sup>) were applied before sowing and N fertilizer (120 kg ha<sup>-1</sup>) applied in early flowering phase.

The applied biofertilizers were two kinds of PSB and two kinds NFB, including *Pseudomonas putida* (strain P13), *Bacillus lentus* (strain P5) and *Azotobacter*, *Azospirillum*, respectively. The bacteria strains were originally isolated from farm soils in Iran. Seeds inoculated with 100 g PSB and 1 lit NFB per hectare before being immediately planted.

#### Yield and yield components

In the full maturity stage, while fruits color became yellow-orange, stem and leaves began to dry, and the seeds became dark green, well rounded fruits were harvested. Ten plants per each treatment were used for final yield analyses. The harvested ripened fruits weighted, and their diameters were measured at the farm. The seeds of each plant were manually collected and dried at shadow in the room temperature. All dried seeds were counted, weighted and measured separately. Some traits such as number of ripened fruits per plant, fruit diameter, 1000-seed weight per fruit, seed and fruit yield were studied.

#### Oil measurement

The oil content of seeds was measured according to Soxhlet method (AOAC, 1980). Measurements of oil yield were calculated from oil content, using the conversion method where the oil

yield was calculated by multiplication of seed yield and seed oil content (Ekin, 2009).

#### Statistical analysis

The collected data from plants in this study was subjected to analysis of variance (ANOVA). Procedure general linear model (GLM) was used for the ANOVA and to test differences between treatments. Means comparison was done through least significant difference (LSD) test by using a statistical analysis system (SAS) statistical package (SAS Institute, 2002).

## RESULTS

### Yield and yield components

Fertilizer treatments had significant effects on the fruit diameter and weight, number of seeds per fruit and seed and fruit yield of pumpkin at 1% probability level. However, there were no significant differences among fertilizer treatments on the number of ripened fruits per plant and 1000- seeds weight (Table 2). Numbers of seeds per fruit were influenced by fertilizer treatments. According to LSD test ( $\alpha = 5\%$ ), maximum seed number per fruit obtained from combined application of microorganisms with 50% organic and chemical fertilizers (391.03 seeds), while there was no significant difference among this compound, NPO (362.72 seeds) and NPC (304.02 seeds). The least seeds number (109.33 seeds) was obtained from un-fertilized treatment (control). Application of all kind of fertilizers significantly increased fruit weight of pumpkin compared to control treatment (no fertilizer). The maximum fruit weight occurred with a mixture of bio fertilizers with a half dose of organic or/and chemical fertilizers (2.85, 2.81 and 2.74 kg) but lowest

**Table 3.** The effect of fertilizer treatments on yield and yield components of pumpkin.

| Treatment | Weight 1000 seed (g) | Number of seed per fruit | Fruit weight (kg) | Fruit diameter (cm) | Number of fruit per plant | Fruit yield (g m <sup>-2</sup> ) | Seed yield (g m <sup>-2</sup> ) |
|-----------|----------------------|--------------------------|-------------------|---------------------|---------------------------|----------------------------------|---------------------------------|
| S         | 122.63 a             | 109.33 d                 | 0.8 c             | 11.67 d             | 1.09 a                    | 1933.7 d                         | 31.26 d                         |
| N         | 133.97 a             | 204.62 c                 | 1.53 b            | 15.49 c             | 1.32 a                    | 3874.9 c                         | 66.03 c                         |
| P         | 129.43 a             | 201.46 c                 | 1.52 b            | 15.17 c             | 1.07 a                    | 3674.6 c                         | 64.77 c                         |
| NP        | 132.33 a             | 204.62 c                 | 1.61 b            | 15.92bc             | 1.41 a                    | 4010.4 c                         | 67.27 c                         |
| C         | 137.57 a             | 236.25 bc                | 1.75 b            | 16.22bc             | 1.04 a                    | 3823.8 c                         | 66.5 c                          |
| O         | 130.07 a             | 185.35 cd                | 1.59 b            | 14.79 c             | 1.26 a                    | 3673.3 c                         | 61.88 c                         |
| CO        | 165.5 a              | 211.44 bc                | 1.99 b            | 15.57bc             | 1.11 a                    | 3400.6 cd                        | 58.22 c                         |
| NC        | 146.03 a             | 258.49 bc                | 2.02 b            | 15.92bc             | 1.16 a                    | 5790.5 ab                        | 74.09 bc                        |
| NO        | 133.5 a              | 259.24 bc                | 1.84 b            | 16.41bc             | 1.2 a                     | 4321.4 bc                        | 70.69 bc                        |
| PC        | 137.07 a             | 226.08 bc                | 1.95 b            | 16.63bc             | 1.19 a                    | 4213.5 bc                        | 69.14 c                         |
| PO        | 126.7 a              | 266.57 bc                | 1.95 b            | 16.19bc             | 1.06 a                    | 4239.1 bc                        | 73.41 bc                        |
| NPC       | 148.3 a              | 304.02 ab                | 2.74 a            | 19.99 a             | 1.36 a                    | 6272.2 a                         | 97.97 ab                        |
| NPO       | 143.63 a             | 367.72 a                 | 2.85 a            | 19.08ab             | 1.57 a                    | 6056.2 a                         | 118.5 a                         |
| NPCO      | 146.37 a             | 391.03 a                 | 2.81 a            | 20.78 a             | 1.3 a                     | 6640.4 a                         | 105.49 a                        |

S, Control; N, NFB, P, PSB; NP, NFB + PSB; C, chemical fertilizer; O, organic fertilizer; CO, 50% organic fertilizer + 50% chemical; NC, NFB + 50% chemical fertilizer; NO, NFB + 50% organic fertilizer; PC, PSM + 50% chemical fertilizer; PO, PSB + 50% organic fertilizer; NPC, NFB + PSB + 50% chemical fertilizer; NPO, NFB + PSM + 50% organic fertilizer; NPCO, NFB + PSB + 50% organic fertilizer+ 50% chemical fertilizer.

**Table 4.** Results of variance analysis of fertilizers treatments on oil percentage and oil yield of seed pumpkin.

| Mean square |    |                |           |
|-------------|----|----------------|-----------|
| SOV         | df | Oil percentage | Oil yield |
| R           | 2  | 95.78          | 126.38    |
| F           | 13 | 9.41           | 222.05    |
| Error       | 26 | 19.22          | 75.69     |
| CV%         | df | 11.2           | 30.3      |

ns, Not significant; \*\*, significant in 1% probability level.

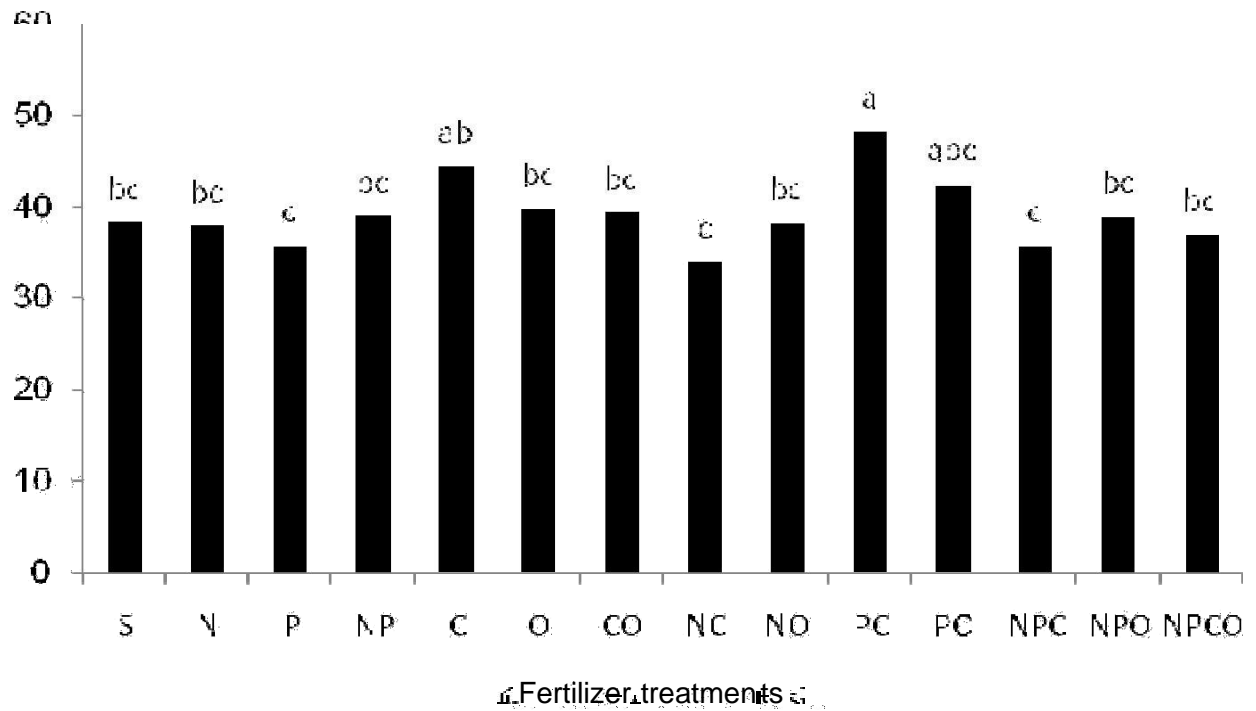
of fruit weight (0.8 kg) occurred in control treatment. There were no significant differences among other treatments. Fertilizer treatments affected the fruit diameter too. NPCO (20.78 cm) and NPC (19.99 cm) treatments led to maximum diameter and the smallest diameter (11.67 cm) was obtained from control treatment (Table 3).

Based on our results, the two types of bio-fertilizers, inoculated pumpkin's seed accompanied by organic fertilizer showed the highest seed yield. No significant difference between these biofertilizers and NPCO (105.49 g m<sup>-2</sup>) and NPC (97.97 g m<sup>-2</sup>) were observed. The least yield (31.26 g m<sup>-2</sup>) was recorded where no fertilizer was added. However there was no significant difference among other treatments. These results indicated that the fertilizer treatments had significant effect on the fruit yield (Table 2). In this study fruit yield varied from 6640.4 to 1933.7 g m<sup>-2</sup>. The highest fruit yield was obtained with applying NPCO (6640.4 g m<sup>-2</sup>), NPC (6272.2 g m<sup>-2</sup>), NPO (6056.2 g m<sup>-2</sup>) and the least fruit

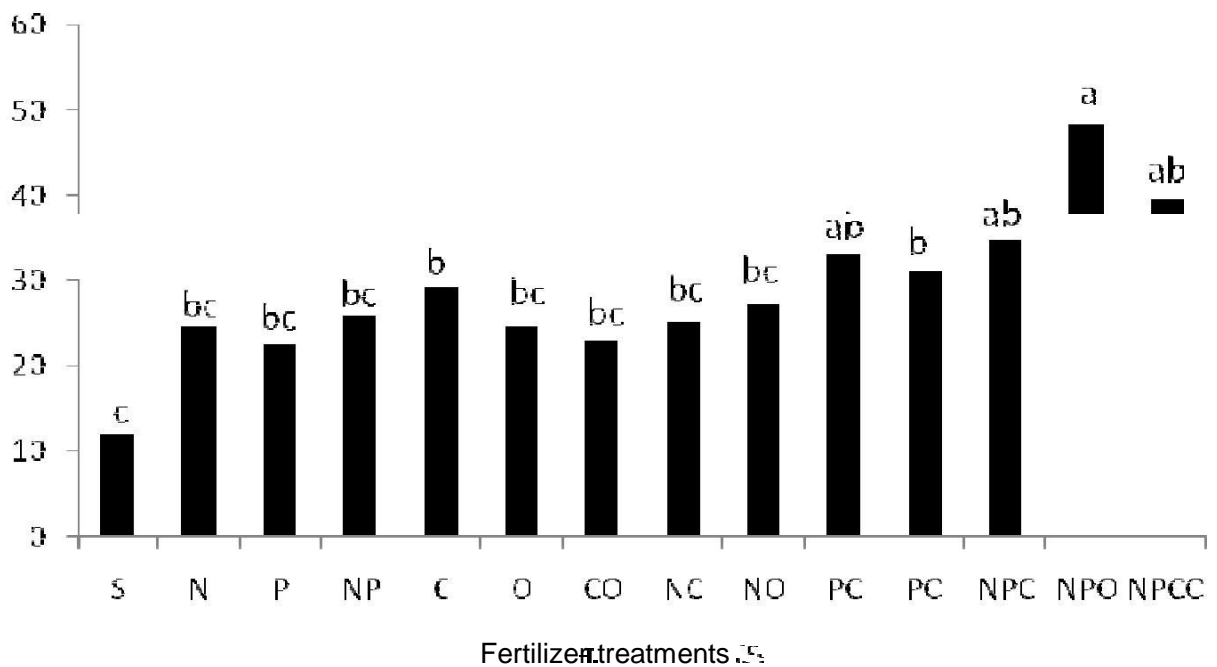
yield (1933.7 g m<sup>-2</sup>) obtained from control treatment (Table 3).

### Oil percentage and yield

ANOVA showed that fertilizers had significant effects on oil percentage and yield (Table 4). The mean of oil content of seeds were between 33.78 to 48.15%. Furthermore, the maximum enhancement of oil percentage (48.15%) was observed in plants which inoculated with phosphate solubilizing bacteria with applying 50% chemical fertilizer. The minimum of oil percentage was observed in application of NFB + chemical fertilizer (33.78%). No significant difference between this fertilizer and others was observed (Figure 1). The highest oil yield was obtained by a mixture of two biofertilizers+ 50% organic fertilizer (48.31 g m<sup>-2</sup>) and the lowest oil yield (12.22 g m<sup>-2</sup>) was resulted with control treatment (Figure 2).



**Figure 1.** The effect of fertilizer treatments on oil percentage of pumpkin. S, Control; N, NFB; P, PSB; NP, NFB + PSB; C, chemical fertilizer; O, organic fertilizer; CO, 50% organic fertilizer + 50% chemical; NC, NFB + 50% chemical fertilizer; NO, NFB + 50% organic fertilizer; PC, PSM + 50% chemical fertilizer; PO, PSB + 50% organic fertilizer; NPC, NFB + PSB + 50% chemical fertilizer; NPO, NFB + PSM + 50% organic fertilizer; NPCO, NFB + PSB + 50% organic fertilizer + 50% chemical fertilizer.



**Figure 2.** The effect of fertilizer treatments on oil yield of pumpkin. S, Control; N, NFB; P, PSB; NP, NFB + PSB; C, chemical fertilizer; O, organic fertilizer; CO, 50% organic fertilizer + 50% chemical; NC, NFB + 50% chemical fertilizer; NO, NFB + 50% organic fertilizer; PC, PSM + 50% chemical fertilizer; PO, PSB + 50% organic fertilizer; NPC, NFB + PSB + 50% chemical fertilizer; NPO, NFB + PSM + 50% organic fertilizer; NPCO, NFB + PSB + 50% organic fertilizer + 50% chemical fertilizer.

**Table 5.** Correlation coefficients between pumpkin traits.

| Trait             |                     |                    |                     |                      |                    |                     |                     |                    |   |
|-------------------|---------------------|--------------------|---------------------|----------------------|--------------------|---------------------|---------------------|--------------------|---|
| Weight 1000 seeds | 1                   |                    |                     |                      |                    |                     |                     |                    |   |
| Number of seeds   | 0.03 <sup>ns</sup>  | 1                  |                     |                      |                    |                     |                     |                    |   |
| Fruit weight      | 0.11 <sup>ns</sup>  | 0.75 <sup>**</sup> | 1                   |                      |                    |                     |                     |                    |   |
| Fruit diameter    | 0.1 <sup>ns</sup>   | 0.71 <sup>**</sup> | 0.65 <sup>**</sup>  | 1                    |                    |                     |                     |                    |   |
| Number of fruits  | -0.06 <sup>ns</sup> | 0.17 <sup>ns</sup> | 0.25 <sup>ns</sup>  | 0.24 <sup>ns</sup>   | 1                  |                     |                     |                    |   |
| Fruit yield       | 0.17 <sup>ns</sup>  | 0.7 <sup>**</sup>  | 0.81 <sup>**</sup>  | 0.8 <sup>**</sup>    | 0.3 <sup>**</sup>  | 1                   |                     |                    |   |
| Seed yield        | 0.13 <sup>ns</sup>  | 0.71 <sup>**</sup> | 0.75 <sup>**</sup>  | 0.75 <sup>**</sup>   | 0.4 <sup>**</sup>  | 0.78 <sup>**</sup>  | 1                   |                    |   |
| Oil percentage    | 0.028 <sup>ns</sup> | -0.2 <sup>ns</sup> | -0.15 <sup>ns</sup> | -0.028 <sup>ns</sup> | 0.05 <sup>ns</sup> | -0.15 <sup>ns</sup> | -0.03 <sup>ns</sup> | 1                  |   |
| Oil yield         | 0.67 <sup>ns</sup>  | 0.6 <sup>**</sup>  | 0.6 <sup>**</sup>   | 0.63 <sup>**</sup>   | 0.38 <sup>**</sup> | 0.59 <sup>**</sup>  | 0.89 <sup>**</sup>  | 0.39 <sup>**</sup> | 1 |

ns, Not significant; \*\*, significant in 1% probability level; \*, significant in 5% probability level.

## DISCUSSION

In general, all the fertilizers showed good effect on seed and fruit yield of pumpkin compared to the control treatment. We can also conclude that application of biological or organic fertilizers alone cannot provide required N and P completely, but applying these fertilizers with the chemical fertilizer will be effective in increasing yield of pumpkin. In combination of fertilizers, essential nutrient elements for plants has been provided, thus dry matter and seed and fruit yield will be increased.

Application of organic fertilizer combined with chemical fertilizer is an important approach to maintaining and improving the soil fertility, increasing fertilizer use efficiency and improving crop yield (Min-gang., 2008). Application of organic fertilizer leads to better root development and consequently, more nutrient uptake. Compost not only slowly release nutrients but also prevents the losses of chemical fertilizers through denitrification, volatilization waste may improve the efficiency of chemical fertilizers (Abedi et al., 2010).

Organic fertilizers have been increased the yield of this plant. On the other hand, nitrogen fixing bacteria inoculations increased plant growth and seed yield with possible mechanisms of N fixation, release phytohormones similar giberellic acid and indole acetic acid (Mahfouz and sharaf-eldin, 2007). P is an essential element for cell division, root development and seed formation (El-gizawy and Mehasen, 2009). Phosphate solubilizing microorganism's secret organic acids and enzymes that act on insoluble phosphates and convert it into soluble form, thus, proving P to plants (Ponmurugan and Gopi, 2006). In addition, the microorganisms involved in P solubilization as well as can enhance plant growth by enhancing the availability of other trace element such as iron (Fe), zinc (Zn), etc (Ngoc son et al., 2006), synthesize enzymes that can modulated plant hormone level, may limit the available iron via siderophore production and can also kill the pathogen with antibiotic (Akhtar and Siddiqui, 2009).

Organic fertilizer may cause maintain beneficial

bacteria in the soil. The stimulatory effect of organic fertilizer on the survival of *Azotobacter* and *pesedomance* might have been exerted directly through its effect on the growth and proliferation of the bacteria, thereby creating a favorable habitat for better survival of the inoculated bacteria (Kumar et al., 2009). Considering, the positive effects mentioned for organic and bio fertilizers, integrated use biological fertilizer with chemical and organic fertilizers increased this plant seed and fruit yield. Similar result in application combination fertilizers were obtained by other scientists (Efthimiadou et al., 2010; Ekin et al., 2009).

There were significant correlations ( $\alpha=1\%$ ) between seed number, fruits weight and diameter (Table 5), mean that more fruit diameter produced more seed per fruit. Developing seeds are the source of auxin, cytokinin and gibberellins synthesis. The physiological roles of endogenous hormones produced in developing seeds are able to pass out of the seed either along the vascular system or by diffusion through the testa which in turn directly stimulate the growth of surrounding tissues of the fruit and indirectly by directing the metabolic transport of photosynthesis required for fruit growth (Ketsa, 1988).

Application of organic fertilizer alone decreased number of seeds per fruit. This could be attributed to deficiency mineral nitrogen in the early development of plant and nitrogen use by soil microbes to break down organic material. These results agree with those of Bashir et al. (2009) for guava, they found that the highest number of seed per fruit was obtained by applying 40 kg farmyard manure + 1 kg each of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O per guava plant. Olaniyi and Odede (2009) showed that the highest seed weight per plant resulted from 30 kg N by 4.5 t ha<sup>-1</sup> of compost.

Application of chemical or organic fertilizers alone produced high seed fat contents because it provides a balanced level of nitrogen for plants, but integrated use of these fertilizers with nitrogen fixing bacteria declined seed fat contents due to increasing nitrogen accessibility. Protein is prior to oil biosynthesis and often negatively correlates with the oil contents (Liu et al., 2009).

Increasing nitrogen level significantly decreased the seed oil content. This decrease might have been resulted from an increase in allocation of assimilates into amino acid, protein and enzyme production in response to nitrogen application (Sayyad-amin and Ehsanzadeh, 2008).

Essential oils are terpenoids based on integral C5 units (isoprenoid). Biologically active isoprenoid requires acetyl-CoA, ATP and Nicotinamide adenine dinucleotide phosphate (NADPH) for synthesis. Hence, the biosynthesis of essential oil is dependent on inorganic P content in the plant (Kapoor et al., 2004). Hence the study suggests that application of PSB in combination with 50% organic and chemical fertilizers has a great potential to increase oil content of pumpkin. Narits (2010) reported that increasing nitrogen level decreased the oil content of winter oil seed rape seed. Ayodele et al. (2006) noted that P fertilizer application tend to increase the oil concentration in the melon seed. Mohamed and Abdu (2004) reported that application of organic manure tend to increasing oil percentage of fennel seed.

Oil yield obtained by multiplying two factors, grain yield and oil percentage (Siam et al., 2008). NPC, NPO and NPCO treatments despite having less oil, because of their high seed yield had the highest oil yield per unit area, compared to other treatments. Control treatment with a minimum amount of seed yield has produced minimum oil yield per unit area (Figure 2). Oil yield is the main purpose of oil seeds cultivation of medicinal pumpkin. Considering that oil percentage is being less influenced by environmental and nutritional factors, the best way to achieve high oil production in plants is increasing their seed yield.

## Conclusion

It is strongly suggested that using biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest plant yield and oil yield. It is clear from results of the present study that 50% of required nitrogen and phosphorus fertilizers could be replaced by bio and organic fertilizers, because bio and organic fertilizers improved the use efficiency of recommended nitrogen and phosphorus fertilizers and reduced the cost of chemical fertilizers, also prevented the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out and it can be help achieving sustainability of farms.

## REFERENCES

- Abdel-Rahman MK (2006). Effect of pumpkin seed (*Cucurbita pepo* L.) diets on benign Prostatic hyperplasia (BPH) chemical and morphometric evaluation in rats. *World J. Chem.*, 1: 33-40.
- Abedi T, Alemzadeh A, Kazemini SA (2010). Effect of organic and inorganic fertilizers on grain yield and protein banding pattern of wheat. *Aus. J. Crop Sci.*, 4(6): 384-389.
- Akhtar MS, Siddiqui ZA (2009). Effect of phosphate solubilizing microorganisms and *Rizobium* sp. On the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. *Afr. J. Biotechnol.*, 8(15): 3489-3496.
- Anjum MA, Sajjad MR, Akhtar N, Qureshi MA, Iqbal A, Jami RA, Hasan MU (2007). Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen. *J. Agric. Res.*, 45(2): 135-143.
- AOAC (1980). Association of official agricultural chemists, Official and Tentative Methods of Analysis, 11th ED., Washington, D.C., USA.
- Applequist WL, Avula B, Schaneberg BT, Wang YH, Khan IA (2006). Comparative fatty acid content of seeds of four *Cucurbita* species grown in a common (shared) garden. *J. Food. Comp. Anal.*, 19: 606-611.
- Awodun MA (2007). Effect of poultry manure on the growth, yield and nutrient content of fluted pumpkin. *Asian J. Agric. Res.*, 1(2): 67-73.
- Ayodele OJ, Omotoso SO, Akinrinsola CO (2006). Phosphorus fertilizer use in melon (Egusi) seed production: effect on yield, oil and protein content and nutrient composition. *Agric. J.*, 1(4): 216-220.
- Azeez JO, Averbek WV, Okorogbona AMO (2010). Differential responses in yield of pumpkin (*Cucurbita maxima* L.) and nightshade (*Solanum retroflexum* Dun.) to the application of three animal manures. *Bioresource Technol. J.*, 101: 2499-2505.
- Azzan NA, Hassan EA, Hamad EH (2009). The chemical constituent and vegetative and yielding characteristics of fennel plants treated with organic and bio-fertilizer instead of mineral fertilizer. *Aust. J. Basic Appl. Sci.*, 3(2): 579-587.
- Bakulin MK, Grudtsyna AS, Pletneva A (2007). Biological fixation of nitrogen and growth of bacteria of the genus *Azotobacter* in liquid media in the presence of Perfluorocarbons. *Appl. Biochem. Microbiol.*, 4: 399-402.
- Banerjee S, Palit R, Sengupta C, Standing D (2010). Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. Isolated from tomato rhizosphere. *Aust. J. Crop Sci.*, 4(6): 378-383.
- Bashir MA, Ahmad M, Salik MR (2009). Manure and fertilizers effect and fruit quality of guava (*Psidium guajava* L.). *J. Agric. Res.*, 47(3): 247-251.
- Chang CH, Yang SS (2009). Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresour. Technol. J.*, 100: 1648-1658.
- Chen J (2006). The combined use of chemical and organic fertilizer and or biofertilizer for crop growth and soil fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. October, Thailand, pp. 16-20.
- Eftimiadou A, Bilalis D, Karkanis A, Froud-Williams B (2010). Combined organic/inorganic fertilization enhance soil quality and increased yield, photosynthesis and sustainability of sweet maize crop. *Aust. J. Crop Sci.*, 4(9): 722-729.
- Ekin Z, Oguz F, Erman M, Ogun E (2009). The effect of *Bacillus* sp. OSU-142 inoculation at various levels of nitrogen fertilization on growth, tuber distribution and yield of potato (*Solanum tuberosum* L.). *Afr. J. Biotechnol.*, 8(18): 4418-4424.
- El-ashry SM, Hanan S, Abd El- Moez MR (2008). Ameliorative effect of organic fertilizers in the presence of mineral fertilizers on lettuce and sorghum yield and their components. *Aust. J. Basic Appl. Sci.*, 2(4): 1246-1257.
- El-Gizawy NB, Mehasen SAS (2009). Response of *Faba bean* to bio, mineral phosphorus fertilizers and foliar application with zinc. *World Appl. Sci. J.*, 6(10): 1359-1365.
- El-Yazid AA, Abou-Aly HA, Mady MA, Moussa SAM (2007). Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. *Res. J. Agric. Biol. Sci.*, 3(4): 274-286.
- Fruhwith GO, Hermetter A (2007). Seed and oil of the styrian oil pumpkin: components and biological activities. *Eur. J. Lipid Sci. Technol.*, 109: 1128-1140.
- Ghaderi FA, Soltani A, Sadeghipour HR (2008). Cardinal temperatures of germination in medicinal pumpkin (*Cucurbita pepo* convar. *Pepo* var. *styriaca*), borage (*Borago officinalis* L.) and black cumin (*Nigella sativa* L.). *Asian J. Plant Sci.*, 7(6): 574-578.
- Gharib F, Moussa LA, Massoud ON (2008). Effect of compost and

- bio-fertilizers on growth, yield and essential oil of sweet marjoram (*Majorana hortensis*) plant. Int. J. Agric. Biol., 10(4): 381-382.
- Gholipouri A, Nazarnead H (2007). The effect of stem pruning and nitrogen levels of on some physicochemical characteristics of pumpkin seed (*Cucurbita pepo* L.). Pak. J. Biol. Sci., 10(20): 3726-3729.
- Gothwal RK, Nigam VK, Mohan MK, Sasmal D, Ghosh P (2007). Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. Appl. Ecol. Environ. Res., 6(2): 101-109.
- Hussain K, Arif M, Anwar-UI-Haq M, Hussain T, Nasim M (2007). Compost for growing plants by applying EM- biofertilier. Pak. J. Agri. Sci., 44(3): 434-442.
- Kapoor R, Giri B, Mukerji KG (2004). Improved growth and essential oil yield and quality in *Foeniculum vulgare* mill on mycorrhizal inoculation supplemented with P-fertilizer. Bioresour. Technol. J., 93: 307-311.
- Kavadia A, Vayenas DV, Pavlou S, Aggelis G (2007). Dynamics of free-living nitrogen-fixing bacterial population in antagonistic conditions. Ecol. Model, 200: 243-253.
- Ketsa S (1988). Effect of seed number on fruit characteristics of tangerine. Nat. Sci., 22: 225- 227.
- Koštílov κZ, Hromádkov κZ, Erbringerov κA (2009). Chemical evaluation of seeded fruit biomass of oil pumpkin (*Cucurbita pepo* L. var. *Styriaca*). Chem. Pap., 63(4): 406-413.
- Krička T, Sito S, Voča N, Barčić J, Jukić Ž, Voča S, Janušić V (2005). Differences in water release rate of hulled and hull-less pumpkin seed. Nat. Resour., 56(3): 153-159.
- Kumar V, Solanki AS, Sharma S (2009) . Yield and economics of withaniasomnifera influenced by dual inoculation *Azotobacter chroococcum* and *Pseudomonas putida*. Turk. J. Biol., 33: 219-223.
- Liu H, Hu C, Sun X, Tan Q, Nie Z, Su J, Liu J, Huang H (2009). Interactive effects of molybdenum and phosphorus fertilizers on grain yield and quality of *Brassica napus*. J. Food Agric. Environ., 7: 266-269.
- Mahfouz SA, Sharaf -Eldin MA (2007). Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). Int. J. Agrophys., 21: 361-366.
- Mao J, Oik DC, Fang X, He Z, Schmidt-Rohr K (2008). Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma, 146: 353-362.
- Mikhailouskaya N, Bogdevitch I (2009). Effect of biofertilizers on yield and quality of long- fibred flax and cereal grains. J. Agron. Res., 7: 412-418.
- Min-gang X, Dong-chu L, Ju-mei L, Dao-zhu Q, Yagi K, Hosen Y (2008). Effect of organic manure application with chemical fertilizers on nutrient absorption and yield of rice in Hunan southern china. China J. Agric. Sci., 7(10): 1245-1252.
- Mohamed MAH, Abdu M (2004). Growth and oil production of fennel (*Foeniculum vulgare* Mill): effect of irrigation and organic fertilization. Biol. Agric. Hortic., 22: 31-39.
- Narits L (2010). Effect of nitrogen rate and application time to yield and quality of winter oilseed rape (*Brassica napus* L. var. *oleifera* sub var. *biennis*). J. Agron. Res., 3: 671-686.
- Ngoc STT, Ngoc DCM, Giang TT (2006). Effect of Bradyrhizobia and phosphate solubilizing bacteria application on soybean in rotational system in the Mekong delta. J. Omonrice, 14: 48-57.
- Nikkhah BR, Khodadadi M, PiryPirivatlo S, Hassanpanah D (2009). The effects of planting methods and head pruning on seed yield and yield components of medicinal pumpkin (*Cucurbita pepo* subsp. *pepo* convar. *pepo* var *styriaca*) at low temperature areas. Pak. J. Biol. Sci., 12(6): 538-541.
- Olaniyi JO, Odedere MP (2009). The effects of mineral N and compost fertilizers on the growth, yield and nutritional values of fluted pumpkin (*Telfairia occidentalis*) in south western Nigeria. J. Anim. Plant Sci., 1: 443-449.
- Peričin D, Radulović L, Trivić S, Dimić E (2008). Evaluation of solubility of pumpkin seed globulins by response surface method. J. Food Eng., 84: 591-594.
- Ponmurugan P, Gopi G (2006). Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron., 5(4): 600-604.
- Rokhzadi A, Asgharzadeh A, Darvish F, Nour-mohammadi G, Majidi E (2008) . Influence of plant growth-promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field condition. Am-Euras. J. Agric. Environ. Sci., 3(2): 253-257.
- Saikia SP, Jain V (2007). Biological nitrogen fixation with non- legumes: an achievable able target or a dogma. Curr. Sci., 92(3): 317-322.
- SAS Institute (2002). The SAS System for Microsoft Windows. Release 8.2. Cary, NC.
- Sayyad-Amin P, Ehsanzadeh P (2008). The effect of nitrogen on seed and oil yield of seven sesame (*Sesamum indicum* L.) genotypes. International Meeting on Soil Fertility and Management and Agroclimatology. Turkey, pp. 581-586.
- Sedghi M, Gholipouri A, Sharifi RS (2008). γ-Tocopherol accumulation and floral differentiation of medicinal pumpkin (*Cucurbita pepo* L.) in response to plant growth regulators. Not. Bot. Hort. Agrobot. Cluj., 36(1): 80-84.
- Shehata MM, El-khawas SA (2003). Effect of biofertilizers on growth parameters, yield characters, nitrogenous components, nucleic acids content, minerals, oil content, protein profiles and DNA banding pattern of sunflower (*Helianthus annus* L. cv. Vedock) yield. Pak. J. Biol. Sci., 6(14): 1257-1268.
- Shirani H, Hajabbasi MA, Afyuni M, Hemmat A (2002). Effect of farmyard manure and tillage system on soil physical properties and corn yield in central Iran. Soil Till. Res., 68: 101-108.
- Siam HS, Abd-El-Kader MG, El-Alla A (2008). Yield and yield components of maize as affected by different sources and application rates of nitrogen fertilizer. Res. J. Agric. Biol. Sci., 4(5): 399-412.
- Tambekar DH, Gulhane SR, Somkuwar DO, Ingle KB, Kanchalwar SP (2009). Potential Rhizobium and phosphate solubilizers as a biofertilizers from saline belt of Akola and Buldhana district (India). Res. J. Agric. Biol. Sci., 5(4): 578-582.
- Warman PR, Rodd AV, Hicklenton P (2009). The effect of MSW compost and fertilizer on extractable soil elements and the growth of winter squash in Nova Scotia. Agric. Ecosyst. Environ., 133: 98-102.
- Xiao CQ, Chi RA, Huang XH, Zhang WX (2008). Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. Ecol. Eng., 33: 187-193.
- Yasari E, Esmaeili AAM, Pirdashti H, Mozafari S (2008). Azotobacter and azospirillum inoculants as biofertilizers in canola (*Brassica napus* L.) cultivation. Asian J. Plant Sci., 7(5): 490-494.