Journal of Applied Horticulture, 16(1): 61-65, 2014

Effect of integrated application of phosphorus and phosphate solubilizing microorganisms on root colonization, productivity and seed quality of *Cucurbita pepo* L.

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Abstract

Phosphorus is a major nutrient and its deficiency limits plant growth of pumpkin (*Cucurbita pepo* L.). The investigation was aimed at studying integrated application of phosphorus on growth and production of pumpkin. Co-inoculation of phosphate solubilizing microorganisms (PSM) (mycorrhiza and bacteria) with and without seed inoculations, and P chemical fertilizer at 0, 25, 50, 75 and 100% of recommended fertilizer were applied in a factorial experiment in randomized complete block design with three replications. Data indicate that PSM and P fertilizer show significant effects on all traits. Maximum oil yield (41.80 g m^{-2}) and linoleic acid (68.30%) were obtained with PSM and 50% of the recommended P fertilizer. Seed yield was significantly increased in response to inoculation of PSM in the presence of low levels of P fertilizer. However, maximum mycorrhizal colonization obtained in 25% recommended P fertilizer. A high level of P fertilizer had a negative effect on the activity of PSM. On the other hand, a low level of phosphorus with PSM has a simulative impact on root colonization and productivity of pumpkin and favoured the activities of PSM.

Key words: *Cucurbita pepo* L., fertilizer, linoleic acid, mycorrhiza fungi, oil percentage, symbiosis.

Introduction

Chemical fertilizer application and inappropriate energy production methods have harmful effects on biological cycle of nutrients and have destroyed stability of farming systems and thus encourage the application of biological fertilizers for restoration (Kannayan, 2002). Nowadays attention to biofertilizers has been increased due to developments of countries, high costs of chemical fertilizers and attention towards sustainable agricultural systems (Ehteshami *et al.*, 2007). Medicinal pumpkin (*Cucurbita pepo* L.) is widely used in traditional and industrial pharmacology (Ghaderi *et al.*, 2008). Pumpkin seed oil contains several essential fatty acids that help to maintain healthy blood vessels, and nerves (Horveth and Bedo, 1998; Applequist *et al.*, 2006). The oil content of pumpkin seed varies from 30-50% (Stevenson *et al.*, 2007) and the composition of fatty acids is dependent on several factors. Pumpkin seed oil, especially when produced organically, is used in pharmacology and alternative medicine (Wagner, 2000).

Phosphorus (P) is one of the major nutrients which limits plant growth when deficient. Most of the soils throughout the world are P deficient (Batjes, 1997) and therefore require P to replenish the P demand by crop plants. P is an essential element for cell division, root development and seed formation (El-gizawy and Mehasen, 2009). It causes early ripening in plants, decreases seed moisture, improves crop quality and is most sensitive nutrient to soil pH. To circumvent the phosphorus deficiency in soils, P fertilizers are applied (Zaidi and Khan, 2006).

Vesicular arbuscular mycorrhiza (VAM) fungi encourage the plant roots to rapidly absorb solubilized phosphorus. VAM fungi play an important role in changing fixed or insoluble P into soluble P, which can be used by plant freely. Thus, inoculation with VAM fungi has been found to increase the availability of P and other nutrients in crop plants. They exert these effects through symbiotic associations with plant roots, colonizing cortical tissues and extending hyphae into the rhizosphere (Hetrick *et al.*, 1996). It is documented that inoculation of sorghum plants with VAM fungi helps to absorb enough micronutrients through chelate formation with sidrophores (Aliasgharzad *et al.*, 2009). Inoculum production from mycorrhizal fungi and using it in proper environmental conditions is a significant environmental friendly way to help plant growth and development through the enhancement of P absorbance (Mehrvarz *et al.*, 2008). The significance of this practice, especially under low fertility conditions, has been evident.

Phosphate solubilizing bacteria are also considered to be among the most effective plant assistants to supply P at a favorable level. Among the soil bacterial communities, *Pseudomonas* sp. and *Bacillus* species could be referred to as the most important strains (Akmakc *et al.*, 2006; Mehrvarz *et al.*, 2008). The population of phosphate solubilizing bacteria depends on cultural activities and different soil properties involving physical and chemical properties, organic matter, and soil phosphorus content (Kim *et al.*, 1989).

This study was designed to evaluate the effects of co-inoculation of VAM fungi with P solubilizing bacteria and effectiveness of different P levels on growth and production of pumpkin.

Materials and methods

Experimental site and design: The study was conducted at the Research Farm of Bu-Ali Sina University, Hamedan, Iran, during growing season of 2010-11. Physical and chemical properties of the site soil are summarized in Table 1. Factorial experiment was carried out based on randomized complete block design (RCBD) with 3 replications. Treatments consisted of co-inoculation of phosphate solubilizing microorganisms (mycorrhiza fungi and phosphate solubilizing bacteria) at two level with (MP_1) and without seed inoculums (MP_0), and phosphorus chemical fertilizer at five levels, 25% (C_1), 50% (C_2), 75% (C_3), 100% of recommended fertilizer (C_4) and control (C_0: no chemical fertilizer). All treatments were applied before planting. The phosphate solubilizing bacteria used for this experiment were supplied by the Green Bio-tech Co., which consisted of *Pseudomonas putida* (strain p13) and *Bacillus lentus* (strain p15). The bacterial strains are endemic to the farm soils of Iran. VAM fungus *Glomus intraradices* (University of Technology, Munich, Germany) was used in the experiments. All seeds were sown soon after the microbial inoculations. P chemical fertilizer as triple super phosphate was utilized as strip under seeds according to experimental treatments. Each plot consisted of 5 rows with 140 cm space between rows and 30 cm distances between plants in the rows. During the different stages of plant growth, practices such as weeding, irrigation and pest control were performed for all plots.

Measurements: During the flowering stage, the percentage of mycorrhizal colonization of roots was measured using a gridline intersection technique, according to Phillips and Hayman (1970). The oil content of seeds was measured according to Soxhlet method (AOAC, 1980). Measurements of oil yield were calculated by multiplying two factors, *i.e.* grain yield and oil percentage (Siam *et al.*, 2008). Also, at the flowering stage, agronomic traits *viz.*, number of leaves per plant, number of branches, number of nodes per plant, and leaf dry weight were measured. Leaf chlorophyll was also measured during phenological stages. At the harvesting time, 2 m^2 from each experimental unit was harvested and based on the fruit number per plant, fruit weight average, seed number per fruit, 100 seed weight, fruit yield and seed yield were determined.

Statistical analysis: The statistical analyses of data were carried out by ANOVA. Comparison of the means was made using LSD (Least Significant Difference) method at the probability level of 5%.

Results

Analyses of variance showed that number of leaves per plant, number of nodes per plant, number of branches, leaf dry weight and leaf chlorophyll were affected by P chemical fertilizer treatment (Table 2). Also, the interaction effect of P solubilizing microorganisms (MP) and P fertilizer (C) for number of leaves per plant, number of nods per plant, number of branches and leaf chlorophyll was significant at 5% probability level (Table 2).

Mean comparisons indicated that highest values of number of leaves per plant (22.33), number of nodes per plant (15) and number of branches (2.63) were obtained with application of mycorrhiza fungi + P solubilizing bacteria and 50% of recommended P fertilizer (MP_1C_2). The lowest value was observed in control (MP_0C_0) (Table 3). Results showed that, 75% P fertilizer produced maximum leaf dry weight, in the flowering stage. With decreasing P chemical fertilizer from 100 to 25% recommended fertilizer, phosphate solubilizing microorganisms significantly increased the leaf chlorophyll content. The maximum leaf chlorophyll (51.65 SPAD) was obtained in phosphate solubilizing microorganisms and 25% recommended P fertilizer plots (MP_1C_1), while the minimum amount of leaf chlorophyll (47.00 SPAD) was obtained in control treatments (MP_0C_0). It seems that P solubilizing microorganisms, *i.e.* mycorrhizal fungi and P solubilizing bacteria are very important components of the treatments that enhance P solubility and other elements.

There was a significant difference in mycorrhiza + P solubilizing bacteria (MP) and P chemical fertilizer treatments in yield and yield components traits (Table 4). Indeed, the interaction effect of inoculation and phosphorus treatments on yield components and fruit yield wasn't significant. The higher value of seed number per fruit (235.72 seeds), 100 seed weight (15.08 g) and fruit weight average (1.77 kg) was obtained in 50% of P recommended fertilizer and the lowest values of seed number per fruit (182.22 seeds) and 100 seed weight (12.66 g) was observed in 100% P recommended fertilizer treatment (C_4). Also, inoculation of P solubilizing microorganisms increased yield components and fruit yield. Moreover, inoculated plants (by Mycorrhiza and bacteria) were more successful than non- inoculated plants that can suggest a synergic effect between bacteria and the fungus (Table 5). The results showed that with increasing use of P chemical fertilizers from 0 to 100%, fruits number per plant increased. According to our findings (Table 5), fruit yield had a range from 5.69 kg m^{-2} in 50% P recommended fertilizer (C_2) to 3.03 kg m^{-2} in control treatment (C_0). Also, seed inoculums had about 35% more fruit yield compared to non-seed inoculums. Moreover, according to the analyses of variance (Table 4), the effects of treatments on pumpkin seed yield were significant. The maximum seed yield of 135.57 g m^{-2} obtained in MP_1C_1 (seed inoculums + 25% P recommended) which was not significantly different from MP_1C_2 and MP_1C_3 treatment (data not shown), and the minimum seed yield of 60.71 g m^{-2} was obtained in MP_0C_0 (control treatment).

Table 1. Soil physical and chemical characteristics of the experimental site

Sample depth (cm)	Soil texture	pH	OC (%)	EC (mMohs/cm)	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
0-30	Silty clay loam	7.7	0.72	1.45	8.2	220	1.06	4	0.94	5

Table 2. Analysis of variance for the effect of phosphorus treatments on growth traits of pumpkin

Variables	df	Number of leaves $plant^{-1}$	Number of nodes $plant^{-1}$	Number of branches	Leaf dry weight	Leaf chlorophyll
Replication	2	0.09^{ns}	0.24^{ns}	0.12^{ns}	0.31^{ns}	0.68^{ns}
MP	4	5.39^{ns}	1.45^{ns}	0.05^{ns}	12.59^{ns}	15.17^{**}
C	1	34.49^{**}	11.38^{**}	1.43^{**}	42.78^{**}	4.68^{*}
MP×C	4	7.04^{*}	6.04^{*}	0.39^{**}	6.26^{ns}	5.70^{*}
Error	18	1.58	1.49	0.05	5.83	1.37
CV (%)		6.85	8.98	12.29	10.24	2.40

ns: non significant;**,* significant at P=0.01 and P=0.05, respectively; MP: mycorrhiza fungi + P solubilizing bacteria; C: P chemical fertilizer.

Table 3. Mean comparisons of the interaction effects of co-inoculation P solubilizing microorganisms and P chemical fertilizer on growth traits of pumpkin

Treatments		Number of leaves plant^{-1}	Number of nodes plant^{-1}	Number of branches	Leaf chlorophyll
Non P Solubilizing Microorganisms application	Non fertilizer	15.33	9.67	1.27	47.00
	25% recommended	16.63	13.50	1.58	47.08
	50% recommended	17.83	14.50	1.83	49.83
	75% recommended	21.86	14.55	2.23	48.45
	100% recommended	18.17	14.83	2.40	48.25
P Solubilizing Microorganisms application	Non fertilizer	16.17	13.00	1.30	49.03
	25% recommended	17.07	14.00	1.42	51.65
	50% recommended	22.33	15.00	2.63	50.40
	75% recommended	21.33	14.75	2.58	48.50
	100% recommended	17.17	12.50	1.83	48.15
LSD 5%		2.16	2.10	0.40	2.01

Table 4. Analysis of variance for the effect of phosphorus treatments on yield and yield components of pumpkin

Variables	df	Seed number per fruit	100 seed weight	Fruit number per plant	Fruit weight average	Seed yield	Fruit yield
Replication	2	414.93^{ns}	2.55^{ns}	0.05^{ns}	0.05^{ns}	122.38^{ns}	2.10*
MP	4	11231.32**	18.67**	0.31**	0.31**	13594.51**	29.62**
C	1	2639.55*	7.18*	0.33**	1.34**	1024.17**	6.09**
MP×C	4	594.41^{ns}	1.41^{ns}	0.02^{ns}	0.08^{ns}	520.96*	1.53^{ns}
Error	18	902.81	2.11	0.01	0.05	175.32	0.55
CV, %		13.98	10.59	9.79	16.13	14.40	15.95

ns: non significant;**,* significant at *P*=0.01 and *P*=0.05, respectively; MP: mycorrhiza fungi + P solubilizing bacteria; C: P chemical fertilizer.

Table 5. Mean comparisons of the main effects of co-inoculation P solubilizing microorganisms and P chemical fertilizer on yield and yield components of pumpkin

Treatments	Seed number per fruit	100 seed weight	Fruit number per plant	Fruit weight average	Fruit yield
PSM application	234.23a	14.52a	1.51a	1.69a	5.67a
No PSM application	195.53b	12.94b	1.31b	1.27b	3.68b
No fertilizer	218.00ab	12.92c	1.14b	1.19c	3.03c
25% of recommended dose	229.46a	14.72ab	1.29b	1.57ab	4.55b
50% of recommended dose	235.72a	15.08a	1.30a	1.77a	5.18ab
75% of recommended dose	208.96ab	13.29bc	1.63a	1.57ab	5.69a
100% of recommended dose	182.22b	12.66c	1.69a	1.31bc	4.91ab

Table 6. Analysis of variance for the effect of phosphorus treatments on mycorrhizal colonization and seed quality of pumpkin

Variables	df	Oil percentage	Oil yield	Mycorrhizal colonization	Oleic acid	Linoleic acid
Replication	2	0.15^{ns}	79.18^{ns}	8.15^{ns}	0.41^{ns}	1.44^{ns}
MP	4	77.95**	2552.83**	11080.33**	11.38**	9.28*
C	1	39.60**	252.73**	138.58**	1.10**	4.19*
MP*C	4	28.40**	99.99^{ns}	79.69^{ns}	0.71**	1.47^{ns}
Error	18	5.74	42.45	28.95	0.15	1.49
CV, %		6.14	18.11	21.11	1.75	1.80

ns: non significant;**,* significant at *P*=0.01 and *P*=0.05, respectively; MP: mycorrhiza fungi + P solubilizing bacteria; C: P chemical fertilizer.

Table 7. Mean comparisons of the main effects of co-inoculation P solubilizing microorganisms and P chemical fertilizer on mycorrhizal colonization and seed quality of pumpkin

Treatments	Oil yield	Mycorrhizal colonization	Linoleic acid
PSM application	45.18a	44.70a	68.13a
No PSM application	26.73b	6.26b	67.02b
No fertilizer	25.33b	17.32b	66.31b
25% of recommended dose	38.60a	29.50a	67.16ab
50% of recommended dose	41.80a	28.33a	68.30a
75% of recommended dose	39.40a	27.33a	68.09a
100% of recommended dose	34.58a	25.16a	68.05a

As seen in Table 6, application of P solubilizing microorganisms and P fertilizer levels had significant effect on quality traits of pumpkin plants. Also, interaction between P solubilizing microorganisms and P fertilizer on oil percentage and oleic acid was significant. Table 7 shows that the highest values of oil yield (41.80 g m^{-2}) and linoleic acid (68.30 %) were obtained with application of 50% P recommended fertilizer. However, the maximum mycorrhizal colonization of 29.50% was obtained in 25% recommended P fertilizer. P fertilizer consumption significantly increased linoleic acid, oil yield and mycorrhizal colonization. The Pumpkin plants showed positive response to P solubilizing microorganism's application for oil yield, mycorrhizal colonization and linoleic acid traits. The highest oil percentage (44.44 %) and oleic acid (23.68 %) were obtained from 50% P fertilizer and inoculated plants (Fig. 1).

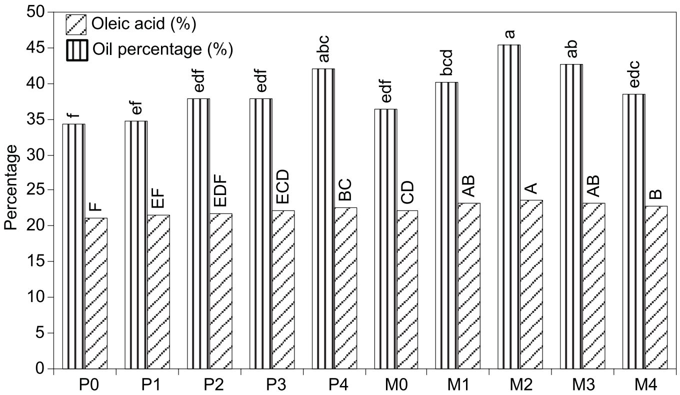

Fig. 1. The interaction effect of P chemical fertilizer levels and co-inoculation of P solubilizing microorganisms on oil percentage and oleic acid of pumpkin.

Discussion

In the present study, it is shown that significant positive effects were obtained with P solubilizing microorganisms and P fertilizer on growth and production traits of pumpkin.

Improved performance of plant with application of P solubilizing microorganisms for growth and yield was probably due to the absorption of more nutrients by roots. It is known that mycorrhiza fungi and P solubilizing bacteria can increase P uptake by plants through their phosphatase activity, a better exploration of the soil by hyphae and the uptake of fixed soil P, which is otherwise unavailable to plant roots. Phosphate solubilizing microorganisms secrete organic acids, and enzymes that act on insoluble phosphates, and convert it into soluble form, thus, providing it to plants (Ponmurugan and Gopi, 2006). In addition, the microorganisms involved in P solubilization can enhance plant growth by enhancing the availability of other trace element such as iron, zinc, etc. (Ngoc *et al.*, 2006; Akhtar and Siddiqui, 2009).

Based on our data, treatments of P solubilizing microorganisms with 50% of P fertilizer increased the number of leaves per plant, number of nodes per plant and number of branches. Similarly it has been shown, that biofertilizer with 50% of chemical fertilizers led to an increase in plant growth, plant height, branch number, fresh and dry weight of safflower in comparison to application of chemical fertilizers alone. It is reported that, soil chemical and biological characteristics were improved by biofertilizers. In addition, due to the use of low doses of chemical fertilizers, agricultural production would be free from contaminants (EL-Habbasha *et al.*, 2007; Salimpour *et al.*, 2010).

Seed yield was significantly increased in response to inoculation of P solubilizing microorganisms combined with P fertilizer compared to control. It seems that application of biofertilizer combined with chemical fertilizer is an important approach in maintaining and improving the soil fertility, increasing fertilizer use efficiency and improving crop yield. Interaction effect was higher with co-inoculation phosphate solubilizing microorganisms and low levels of chemical fertilizer. Thus, it can be concluded that the high rates of P chemical fertilizers application, lead to antagonistic interaction with mycorrhiza and bacteria.

In inoculated plants (by mycorrhiza and bacteria), the leaf chlorophyll content was significantly increased compared to non-inoculated plants. In this respect, it is indicated that mycorrhizal fungi symbiosis increased the rate of photosynthesis (Augé, 2001). Concentration of chlorophyll in mycorrhizal plants was higher than control plants resulting production of larger grains and enhanced economical yield. It is reported that grain yield and dry matter production in barley increased by utilization of P solubilizing bacteria in the presence P chemical fertilizer. Advantages of P solubilizing bacteria on enhanced plant growth have been previously observed on different crops (Kim *et al.*, 1998; Fernandez *et al.*, 2007).

In this study, co-application of P solubilizing microorganisms and P fertilizer (up to 50% recommended dose) reduced the seed oil percentage and oleic acid. Also, the oil percentage and oleic acid were increased with increase in P fertilizer application from 0 to 100% of recommended dose. Biologically active isopernoid 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). Our results suggest that application of P solubilizing microorganisms in combination with 50% P chemical fertilizers has a great potential to increase oil content of pumpkin. Application of P solubilizing microorganisms and P fertilizer produced high oil yield. Oil yield is the main purpose of oil seeds cultivation of medicinal pumpkin.

Based on our findings, with increase in seed oil content, linoleic acid increased, indicating a positive correlation between oil content and linoleic acid (Gholipouri and Nazarnejad, 2007). The relative amount of oleic acid is always negatively correlated with the relative amount of linoleic acid (Murkovic *et al.*, 1996). This is due to the precursor-product relationship of these two fatty acids. Results showed that amount of pumpkin linoleic acid were higher than oleic acid. The high content of linoleic acid is an important nutritional aspect of pumpkin seed oil. Linoleic acid is an essential fatty acid for humans as it is required for the formation of cellular membranes, vitamin D and various hormones.

Based on our findings it seems that seed inoculation with P solubilizing microorganisms, in general, has a simulative impact on growth, yield and its components, productivity and seed quality of pumpkin. Moreover, there is a synergic relationship between bacteria and fungi. Further, data suggest that low levels of phosphorus fertilizer favour the activities of fungi and bacteria.

References

Abou El-Yazeid, A. and H.E. Abou-Aly, 2011. Enhancing growth, productivity and quality of tomato plants using phosphate solubilizing microorganisms. *Austral. J. Basic Appl. Sci.,* 5: 371-379.

Akhtar, M.S. and Z.A. Siddiqui, 2009. Effect of phosphate solubilizing microorganisms and *Rizobium* sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. *African J. Biotechnol.,* 8: 3489-3496.

Akmakc, C.I.R., F. Donmez, A. Aydin and F.S. Ahin, 2006. Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem.,* 38: 1482-1478.

Aliasgharzad, N., E. Shirmohamadi and S. Oustan, 2009. Sidrophores production by mycorrhizal sorghum roots under micronutrient deficient condition. *Soil Environ.,* 28: 119-123.

AOAC, 1980. Association of Official Agricultural Chemists, *Official and Tentative Methods of Analysis.* Eleventh Edition, Washington DC, USA.

Applequist, W.L., B. Avula, B.T. Schaneberg, Y.H. Wang and I.A. Khan, 2006. Comparative fatty acid content of seeds of four Cucurbita species grown in a common (shared) garden. *J. Food Comp. Anal.,* 19: 606-611.

Augé, R.M., 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza,* 11: 3-42.

Batjes, N.H. 1997. A world dataset of derived soil properties by FAO-UNESCO soil unit for global modelling. *Soil Use Mgt.,* 13: 9-16.

Ehteshami, S.M.R., M. Aghaalikhani, K. Khavazi and M.R. Chaichi, 2007. Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (*Zea mays* L.) under water deficit stress. *Pakistan J. Biol. Sci.,* 10: 3585-3591.

El-Gizawy, N.B. and S.A.S. Mehasen, 2009. Response of faba bean to bio, mineral phosphorus fertilizers and foliar application with zinc. *World Appl. Sci. J.,* 6: 1359-1365.

El-Habbasha, S.F., M. Hozayn and M.A. Khalafallah, 2007. Integration effect between phosphorus levels and biofertilizers on quality and quantity yield of faba bean (*Vicia faba*) in newly cultivated sandy soils. *Res. J. Agr. Biol. Sci.,* 3: 966-971.

Ghaderi, F.A., A. Soltani and H.R. Sadeghipour, 2008. Cardinal temperatures of germination in medicinal pumpkin (*Cucurbita pepo*), borage (*Borago officinalis* L.) and black cumin (*Nigella sativa* L.). *Asian J. Plant Sci.,* 7: 574-578.

Gholipouri, A. and H. Nazarnejad, 2007. The effect of stem pruning and nitrogen levels on some physico-chemical characteristics of pumpkin seed (*Cucurbita pepo* L.). *Pakistan J. Biol. Sci.,* 10: 3726-3729.

Horveth, S. and Z. Bedo, 1998. Another possibility in treatment of hyperlipidaemia with peponen of natural active substance. *Mediflora*, 89: 7-8.

Hetrick, B.A.D., G.W.T. Wilson and T.C. Todd, 1996. Mycorrhizal response in wheat cultivars: Relationship to phosphorus. *Can. J. Bot.,* 74: 19-25.

Kannayan, S. 2002. *Biofertilizers for Sustainable Crop Production, Biotechnology of Biofertilizers*. Narosa Publishing House, New Delhi, India.

Kapoor, R., B. Giri and K.G. Mukerji, 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill on mycorrhizal inoculation supplemented with P-fertilizer. *Biores. Technol. J.*, 93: 307-311.

Kim, K.Y., D. Jordan and G.A. McDonald, 1989. Effect of phosphate-solubilizing bacteria (PSB) and VAM on tomato growth and soil microbial activities. *Biol. Fert. Soils,* 26: 79-87.

Kim, K.Y., G.A. McDonald and D. Jordan, 1998. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biol. Fert. Soils,* 26: 79-87.

Mehrvarz, S., M.R. Chaichi and H.A. Alikhani, 2008. Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barley (*Hordeum vulgare* L.). *Amer. Eurasian J. Agri. Environ. Sci.,* 3: 822-828.

Murkovic, M., A. Hillebrand, J. Winkler, E. Leitner and W. Pfannhauser, 1996. Variability of fatty acid content in pumpkin seeds (*Cucurbita pepo* L.). *Z Lebensm Unters Forsch,* 203: 216-219.

Ngoc, S.T.T., D.C.M. Ngoc and T.T. Giang, 2006. Effect of bradyrhizobia and phosphate solubilizing bacteria application on soybean in rotational system in the Mekong delta. *J. Omonrice,* 14: 48-57.

Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.,* 55: 157-160.

Ponmurugan, P. and G. Gopi, 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *Agron. J.,* 5: 600-604.

Salimpour, S., K. Khavazi, H. Nadian, H. Besharati and M. Miransari, 2010. Enhancing phosphorous availability to canola (*Brassica napus* L.) using P solubilizing and sulfur oxidizing bacteria. *Austral. J. Crop Sci.,* 4: 330-33.

Siam, H.S., M.G. Abd-El-Kader and A. El-Alia, 2008. Yield and yield components of maize as affected by different sources and application rates of nitrogen fertilizer. *Res. J. Agr. Biol. Sci.,* 4: 399-412.

Stevenson, D.G., F.G. Eller, L. Wang, J.L. Jane, T. Wang and G.E. Inglett, 2007. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J. Agr. Food Chem.,* 55: 4005-4013.

Wagner, F.S. 2000. The health value of strain pumpkin seed oil-Science and fiction. *Cucurbit Genet. Coop. Rept.,* 23: 122-123.

Zaidi, A. and M.S. Khan, 2006. Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on green gram-bradyrhizobium symbiosis. *Tur. J. Agr. For.,* 30: 223-230.

Received: January, 2014; Revised: March, 2014; Accepted: April, 2014