Middle-East Journal of Scientific Research 14 (7): 940-946, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.14.7.2124

Effects Of Phosphate Solubilizing Microorganisms and Plant Density on Seed Yield and Essential Oil Content of Anise (*Pimpinella anisum*)

Arameh Zand, Mohammad Taghi Darzi and Mohammad Reza Haj Seyed Hadi

Department of Agronomy, Roudehen Branch, Islamic Azad University, Roudehen, Iran

Abstract: The aim of this study was to investigate the effects of phosphate solubilizing microorganisms and plant density on seed yield and essential oil content of anise (*Pimpinella anisum)*. The experiment was conducted during the growing season of 2009 at the Experimental Station of the Agricultural Research Center of Varamin, Iran. Treatments consisted of phosphate solubilizing microorganisms with three levels (P_1 = control, P_2 = seed inoculation and P_3 = seed inoculation + spraying on the plant base at stem elongation stage) and plant density at four levels (67, 34, 23 and 17 plants/m^2). The present results indicated that phosphate solubilizing microorganisms had positive effects in all measured traits, especially when it used at two times (at seed inoculation + spraying on the plant base at stem elongation stage or A_4 treatment). The highest seed yield and essential oil content in seeds was obtained By using17 plants/m^2.

Key words: Phosphate solubilizing microorganisms · Anise · Yield · Biofertilizer

INTRODUCTION

Anise (*Pimpinella anisum*) is a herbaceous annual plant, which is native to Mediterranean region. Anise is primarily grown for its fruits, commercially called seeds. The anise seeds have essential oil as an active substance, while anehtole is the most important constituent of anise, which is used in pharmaceutical, food, perfumery and flavouring industry [1, 2].

Phosphorus (P) is one of the major essential macronutrients for biological growth and development [3]. Phosphorus is added to soil as inorganic phosphates. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is immobilized rapidly after application [4] and becomes unavailable to plants [5]. Different parameters such as soil pH, calcium concentration, proportion of organic matter, type and proportion of clay, soil moisture, soil texture, root density and exudates can affect the availability of soil P to the plant [6, 7]. Therefore, P is often a limiting nutrient in agricultural soils.

Current trends in agriculture are centered on reducing the use of chemical fertilizers and providing plant nutrition by adding biofertilizers such as Phosphate solubilizing microorganisms (PSM) to the soil [8].

Phosphate solubilizing microorganisms (10% of total soil microorganisms), which include a large number of soil micro-flora [9, 10], largely include bacteria and fungi viz. some of the species of *Bacillus, Pseudomonas, Penicillium* and *Aspergillus* [11, 12]. PSM can solubilize and mineralize P from inorganic and organic pools of total soil P and may be used as inoculants to increase P-availability to plants [13-15] and also have the capacity to increase the growth and yield of crop plants [4, 16, 17] besides reducing disease severity [18, 19].

Pseudomonas spp. has been shown to be well adapted for growth and able to compete effectively for sites in the rhizosphere where nutrients are available [20, 21]. According to Dileep Kumar [22], seed bacterization with these plant growth-promoting rhizobacteria for disease suppression and increased plant growth and yield is fast emerging.

Plant density per unit area is one of the important yield determinants of crops. Plant density is an efficient management tool for maximizing grain yield by increasing the capture of solar radiation within the canopy [23]. An optimum plant population for maximum economic yield exists for all crop species and varies with cultivar and environment [24].

Corresponding Author: Arameh Zand, Department of Agronomy, Roudehen Branch, Islamic Azad University, Roudehen, Iran.

High plant density may increase relative humidity within the canopy and increase the duration of leaf wetness by reducing air movement and sun light penetration [25, 26]. Thus, plant density could have significant impact on plant disease incidence [25, 27]. Several studies have been conducted on the effect of plant density on yield and essential oil percentage in some medicinal plants such as *Artemisia annua* [28], *Cuminum cyminum* [29], *Cychorium intybus* [30], *Pimpinella anisum* [31] and *Matricaria chamomilla* [32].

Some studies has been carried out on responses of anise to different biofertilizers but providing nutrients based on PSM has not been studied well. So, the main objective of this study was to investigate the effects of PSM and plant density on seed yield and essential oil of anise.

MATERIALS AND METHODS

Field Experiment: The present study was conducted during the growing season of 2009 at the Experimental Station of the Agricultural Research Center of Varamin, Iran (Latitude: 35° 21′ N; Longitude: 51° 38′ E; Elevation: 957 m). The soil of the experimental region was loamy clay with pH 7.6 (Table 1).

The experimental design was factorial, based on Randomized Complete Block Design (RCBD) with three replications. Treatments consisted of PSM with three levels (P1= control, P2= seed inoculation, P3= spraying at the base of plants at the start of stem elongation phase) and plant density at four levels (D_1=17, D2= 23, D3= 34 and D4= 67 plant/m^2).

Inoculation was carried out by dipping the anise seeds in the cells suspension of 10^8 CFU/ml for 15 min (Morgenstern and Okon 1987).

Before the planting time, the field was ploughed and harrowed thoroughly up to the depth of 30 cm and leveled. Each experimental plot was 3 m long and 1.5 m wide with the total area of 4.5 m^2. Anise seeds were obtained from the Research Center of Medicinal Plants, Isfahan, Iran.

Planting was done manually, 3 cm depth and in rows with 30 cm apart on 21 April 2009. Three weeks after sowing, the seedlings were thinned. Irrigation furrows with uniform slopes were constructed in each experimental plot. A one-time irrigation was applied immediately after sowing for uniform emergence.

Weeds were controlled manually. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.

Measurements: Final seed yield and yield components were measured from 15 plants. Plants were selected randomly from 2 inner rows in each plots. Characters consisted of plant height, umbel number per plant, seed number per umbel, weight of 1000 seeds, seed yield and essential oil content in seeds.

At the beginning of the flowering period, plant height was measured for each plot using a ruler (±0.1 cm) from the base to the tip of plant.

To determine the amount of essential oil, a sample of 100 g of seeds were mixed with 500 ml of tap water in a ?ask and the water was distilled for 3 h using a Clevenger-type apparatus. The oil content was measured by following the protocol of Letchamo and Marquard [33], based on ml oil per 100 g seed.

Statistical Analysis: All data were subjected to the statistical analysis (one-way ANOVA) using SAS software (SAS Institute Inc, 2002). Means of comparisons were performed by Duncan's Multiple Range Test (DMRT) at 5% probability level. Data were transformed when necessary before analysis to satisfy the assumptions of normality. However, any values mentioned in this section refer to the original data of present experiment.

RESULTS

Plant Height: Plant height did not response to PSM treatments. But, plant density had significant effects on plant height (Table 2). Mean comparison, also, did not show significant differences between various levels of PSM. Mean comparison showed significant differences between various levels of plant density. D_1 (17 plants/m^2) caused the plant to reach the highest height (45.9 cm).

Table 1: Results of soil tests used in research

Year	Soil depth cm	pH	EC dSm^{-1}	T.N.V mg/kg	O.C mg/kg	Soil Texture	N $mgkg^{-1}$	P $mgkg^{-1}$	K $mgkg^{-1}$	Fe $mgkg^{-1}$
2009	0-30	7.6	3	17	0.81	Loam, clay	0.037	9.5	324	5.2

Table 2: Analysis of variance

		Ms						
S.O.V	df	Plant height	Number of umbel per plant	Number of seeds per umbel	1000-Seed weight	Biological yield	Seed yield	Essential oil content
Replication	2	8.82 ns	10.13ns	137.9ns	0.023ns	11043ns	3804ns	0.005ns
Phosphate Solubilizing Microorganisms (P)	3	27.35ns	35071**	2253**	0.046ns	206238**	72133**	4.11**
Density plant (D)	2	10.05**	288.58**	1667**	0.056ns	95513**	77233**	1.29*
(P×D)	6	14.11ns	33.99**	159.16**	0.031ns	163970**	14839ns	2.16**
Error	22	5.68	8.69	38	0.026	8410.06	18238	0.01
C.V		5.55%	8.66%	3.91%	10.13%	16.49%	17.79%	1.98%

**,*, ns, respectively, indicating no significant difference is significant at the 5 and 1 percent.

Table 3: The main effect of comparison

Factor levels	Plant height	Number of umbel per plant	Number of seeds per umbel	Biological yield	Seed yield	Essential Oil content
D1	45.9[a]	40.17[a]	138.68[d]	13001.87[a]	1324.12[a]	6.22[a]
D2	42.47[b]	31.45[b]	150.8[c]	8095.6[b]	674.97[b]	5.74[b]
D3	42.3[b]	38.04[a]	167.41[b]	6065.5[c]	562.84[bc]	4.61[d]
D4	41.13[b]	26.52[c]	173.44[a]	5396.8[d]	473.49[c]	5.47[c]
P1		30.24[b]	144.92[c]	7567.3[c]	630.35[b]	5.3[b]
P2		32.32[b]	159.59[b]	7847.5[b]	738.96[b]	5.35[b]
P3		39.58[a]	168.242[a]	9005[a]	907.26[a]	5.89[a]

Average that have common letters are not significantly different. Treatments consisted of PSM with three levels (P1= control, P2= seed inoculation, P3= spraying at the base of plants at the start of stem elongation phase) and *plant density* at four levels (D_1=17, D2= 23, D3= 34 and D4= 67 plant/m^2)

Number of Umbel per Plant: Both, PSM and plant density treatments had significant effects on number of umbel per plant (Table 2). The lowest number of umbel per plant (24.3) was obtained under P_1 (Control) while the highest number of umbel (39.6) was obtained under P_3 (seed inoculation +spraying at the base of plants at the start of stem elongation phase). Also, mean comparison indicated significant differences between various levels of plant density. 17 plants per m^2 (D1) had the most positive effect on this trait (Table 3). Although, there were not significant differences between D1 and D3. The lowest umbel per plant observed at D_4 treatment.

1000 Seeds Weight: During the present experiment, the one thousand seed weight was not significantly influenced by the treatments (Table 2). Also, Mean comparisons did not show any differences between PSM and density levels.

Number of Seeds per Umbel: All treatments and interaction between various levels of treatments caused significant effects on number of seeds per umbel (Table 2). According to the mean comparison results, the highest number of seeds per umbel (173.4) was found in D_4 (67 plants/m^2) and the lowest seed number (138.7) was related to D1 (17 plants/m^2). Mean comparison showed significant differences between various levels of PSM. P_3 (seed inoculation +spraying at the base of plants at the start of stem elongation phase) caused the plant to reach the highest seed number per umbel (168.24)(Table 3). On the basis of interaction effects, D4P3 had the most positive effects on seed number per umbel. It means by increasing plant density, the effects of PSM on seed number will be more and because of that, this traits increased significantly.

Biological Yield: Plants grown under the D_1 (17 plants/plant) treatment showed a higher biomass production compared to plants grown under the D_2, D_3 and D_4 treatments. This was consistent with higher plant height too (Table 3). Mean comparison showed significant differences between various levels of PSM. P_3 treatment (seed inoculation + spraying on the plant base at stem elongation stage) caused the greatest biomass production (Table 3).

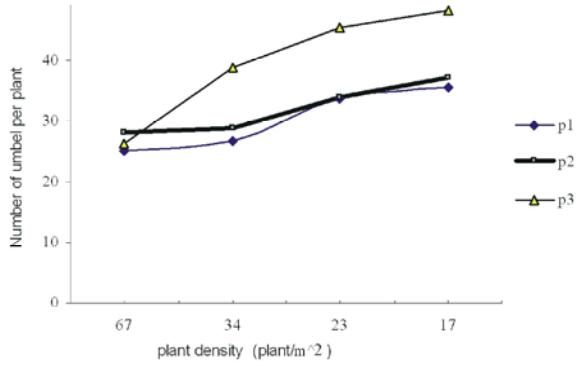

Fig. 1: Effect Density plant and Phosphate Solubilizing Microorganisms on Number of umbel per plant

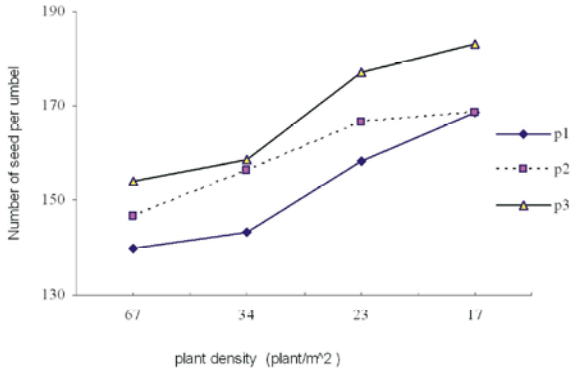

Fig. 2: Effect Density plant and Phosphate Solubilizing Microorganisms on Number of seed per umbel

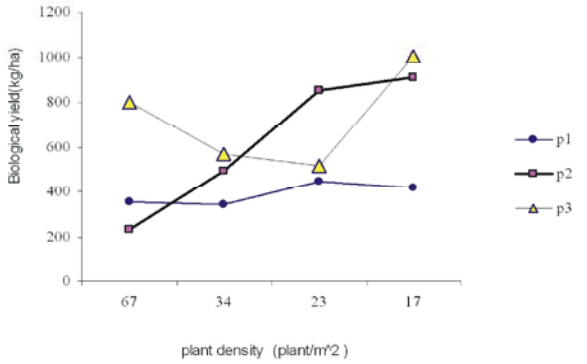

Fig. 3: Effect Density plant and Phosphate Solubilizing Microorganisms on Biological yield

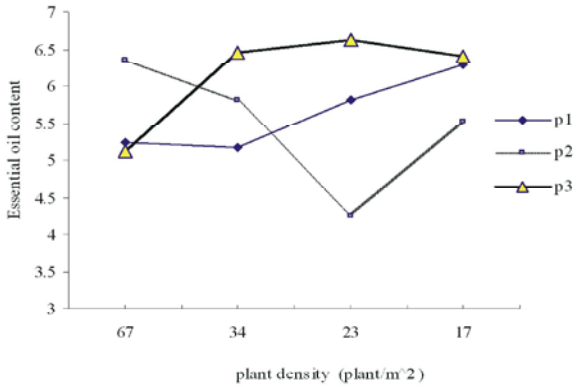

Fig. 4: Effect Density plant and Phosphate Solubilizing Microorganisms on Essential oil content

Seed Yield: Results showed that PSM and plant density had signi?cant effects on the seed yield. Mean Comparison showed that seed yield varied between 1324.12 and 473.49 kg/ha (Table 3), which was obtained from D_1 and D_4, respectively.Seed yield in response to PSM showed the highest increase compared to control. The high seed yield of anise under P_3 treatment might be due to a higher number of umbel per plant and the highest number of seeds per umbel (Table 3). Relationship between number of umbel per plant and seed yield showed that linear regression could explain their relationship.

Essential Oil Content: Essential oil content of seeds respond, significantly, to PSM and plant density treatments. Also, interaction between treatments had significant effects on this trait. The highest essential oil percentage (%6.22) obtained at D_1 (17 plants/m^2). D_2 (34 plants/m^2) caused the lowest essential oil content in seeds (%4.61) (Table 3). Mean comparison for PSM treatments showed that at P3 (seed inoculation +spraying at the base of plants at the start of stem elongation phase) seeds had the highest essential oil (%5.89). but, there were not differences between P1 (control) and P2 (seed inoculation) treatments (Table 3).

DISCUSSION

According to the present analysis, phosphatic biofertilizers have promoted flowering and increased umbel number per plant by enhancing the phosphorus content and the rate of photosynthesis [34]. The present result were derived from the improvement of phosphate solubilizing microorganisms' activities in soil at the third treatment level (seed inoculation + spraying on plant base at stem elongation phase), which are in agreement with the previous studies carried out on the borage plant [35]. Effect of phosphate solubilizing bacteria on the biological yield was due to increased phosphorus uptake [34, 35, 36]. The result of present work are in agreement with the reports of Omar [37] on *Triticum aestivum*, Ratti *et al.* [35] on *Cymbopogon martini* and Rashmi *et al.* [38] on *Ocimum gratissimum*.

Phosphatic biofertilizer promoted seed yield through the enhancement of yield attributes. These result are in agreement with the investigation of Singh and Kapoor [39] on *Vigna radiata* and *Triticum aestivum* and Shaalan [35, 36] on *Borago officinalis* and *Nigella sativa*.

Plant density has a significant effect on plant height [40]. At densities higher than optimal, less light receive by each plant and hence dry matter production per plant decreases [41].

According to ATA [42] by increasing plant density, oil content of *Nigella sativa* L. is reduced. In another experiment the effect of density on yield and yield components of *Coriandrum sativum* L. were evaluated. The results showed that by increasing plant density per unit area, number of umbrella per plant and seed yield decreased linearly [43]. Also, Shalaby *et al.* [44] found that plant density has a significant effects on *Thymus vulgaris*.

CONCLUSIONS

It is clear from the present study that biofertilizers successfully manipulate the growth of anise, resulting in beneficial changes in yield and yield components. The highest biological and seed yield was obtained by using phosphate solubilizing microorganisms Maximum biological and seed yield and essential oil content in seeds were observed by using PSM at two times (seed inoculation +spraying at the base of plants at the start of stem elongation phase). Also, plant density had significant effects on yield and essential oil. The highest seed yield and essential oil were obtained at D4 (17 plants/m^2). D4P3 caused the highest essential oil content in seeds.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Behnam Hossini for providing technical assistance to undertake this research project.

REFERENCES

1. Tuncturk, M. and B. Yildirim, 2006. Effect of seed rates on yield and yield components of anise (*Pimpinella anisum*). Indian J. Agric. Sci., 76(11): 679-681.
2. Ozkan, M.M. and J.C. Chalchat, 2006. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. Ann. Microbiol, 56(4): 353-358.
3. Fernandez, L.A., P. Zalba, M.A. Gomez and M.A. Sagardoy, 2007. Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol. Fert. Soils, pp: 43805-809. R.S. Gadagi and T. Sa, 2002. New Isolation Method for Microorganisms.
4. Rodriguez, H. and R. Fraga, 1999. Phosphate-solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17: 319-339.
5. Singh, S. and K.K. Kapoor, 1994. Solubilization of insoluble phosphates by bacteria isolated from different sources. Environ. Ecol., 12: 51-55.
6. Tisdale, S.L., W.L. Nelson, J.D. Beaton and J.L. Havlin, 1993. Soil Fertility and Fertilizers. 5th ed., Mcmillon Publishing Co., New York, pp: 561-576.
7. Barber, S.A., 1995. Soil Nutrient Bioavailability. John Wiley and Sons Inc., pp: 23-28.
8. Gyaneshwar, P., G. Naresh Kumar, L.J. Parekh and P.S. Poole, 2002 Role of soil microorganisms in improving P nutrition of plants. Plant Soil, 245: 83-93.
9. Whitelaw, M.A., T.Y. Harden and G.L. Bender, 1997. Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. Nov. Aust. J. Soil Res., 38: 291-300.
11. Tilak, K.V.B.R., 1991. Bacterial Fertilizers. Publications and information Division ICAR, New Delhi, pp: 66.
12. Tilak, K.V.B.R., N. Ranganayak, K.K. Pal, R. De, A.K. Saxena, C.S. Nautiyal, S. Mittal, Tripathi.
13. Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol., 28: 897-906.
14. Illmer, P., A. Barbato and F. Schinner, 1995. Solubilization of hardly soluble AlPO with P-solubilizing microorganisms. Soil Biol. Biochem., 27: 265-270.
15. Whitelaw, M.A., T.J. Harden and K.R. Helyar, 1999. Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. Soil Biol. Biochem., 31: 655-665.
16. Gupta, S.C. and S.L. Namdeo, 1997. Effect of Rhizobium, phosphate solubilizing bacteria and FYM on nodulation, grain yield and quality of chickpea. Indian J. Pulse Res., 10: 171-174.
17. Ozgonen, H., M. Bicici and A Erkilic, 1999. The effect of salicylic acid andendomycorrhizal fungus G. intraradices on plant development of tomato and Fusarium wilt caused by *Fusarium oxysporum f. sp.lycopersici*. Turk. J. Agric. For., 25: 25-29.

18. Weller, D.M., 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol., 26: 379-407.
19. Siddiqui, Z.A. and I. Mahmood, 1999. Role of bacteria in the management of plant parasitic nematodes. A Review. Bioresour. Technol., 69: 167179.
20. Bowen, G.D. and A.D. Rovira, 1999. The rhizosphere and its management to improve plant growth. Adv. Agron., 66: 1-102.
21. Kloepper, J.W., C.M. Ryu and S. Zhang, 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology, 94: 1259-1266.
22. Dileep Kumar, B.S., 1998. Disease suppression and crop improvement through fluorescent pseudomonads isolated from cultivated soils. World J. Microbiol. Biotechnol., 14: 735-741.
23. Monneveux, P., P.H. Zaidi and C. Sanchez, 2005. Population density and low nitrogen affects yield. Associated Traits in Tropical Maize. Crop Sci., 45(2): 103-106.
24. Bruns, H.A. and H.K. Abbas, 2005. Ultra-High plant populations and nitrogen fertility effects on corn in the Mississippi Valley. Agron. J., 97(4): 1136.
25. Burdon, J.J. and G.A. Chilvers, 1982. Host density as a factor in plant diseased ecology. Annu. Rev. Phytopathol., 20: 143-166.
26. Tu, J.C., 1997. An integrated control of white mold (*Sclerotinia sclerotiorum*) of beans, with emphasis on recent advances in biological control. Bot. Bull. Acad. Sin., 38: 73-76.
27. Copes, W.E. and H. Scherm, 2005. Plant spacing effects on microclimate and Rhizoctonia web blight development in container-grown Azalea. Hortic. Sci., 40: 1408-1412.
28. Ram, M., M.M. Gupta, S. Dwivedi and S. Kumar, 1997. Effect of plant density on the yields of artemisinin and essential oil in *Artemisia annua* cropped under low input cost management in north-central India. Planta Med., 63(4): 372-374.
29. Hashemi, P., A. Yarahmadi, K.H. Azizi and B. Sabouri, 2007. Study of the effects of N fertilization and plant density on the essential oil composition and yield of Cuminum cyminum L. seeds by HS–SME. Chromatographia, 67(3): 253-257.
30. Taheri, A.M., J. Daneshian, S.A.R. Valadabadi and H. Aliabadi Farahani, 2008. Effects of water deficit and plant density on morphological characteristics of chicory (*Cichorium intybus* L.). 5th International Crop Science Congress and Exhibition, pp: 26.
30. Zehtab-Salmasi, S., A. Javanshir, R. Omidbaigi, H. Alyari and K. Ghassemigolezani, 2001. Effects of water supply and sowing date on performance and essential oil production of anis (*Pimpinella anisum* L.). Act. Agro. Hun., 49(1): 75-81.
32. Hadj Seyed, Hadi M., G. Noormohammadi, J.M. Sinaki, N. Khodabandeh, N. Yasa and M.T. Darzi, 2004. Effects of planting time and plant density on flower yield and active substance of Chamomile (*Matricaria chamomilla* L.). 4th International Crop Science Congress, pp: 280.
33. Letchamo, W. and R. Marquard, 1993. The pattern of active substances accumulation in chamomile genotypes under different growing conditions and harvesting frequencies. Acta Hort., 331: 357-364.
34. Ratti, N., S. Kumar, H.N. Verma and S.P. Gautam, 2001. Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martinii var. motia* by rhizobacteria, AMF and *Azospirillum* inoculation. Microbiol. Res., 156: 145-149.
35. Shaalan, M.N., 2005a. Effect of compost and different sources of biofertilizers, on borage plants (*Borago officinalis* L.). Egyptian J. Agric. Res., 83(1): 271-284.
36. Shaalan, M.N., 2005b. Influence of biofertilizers and chicken manure on growth, yield and seeds quality of *Nigella sativa* L. plants. Egyptian J. Agric. Res., 83(2): 811-828.
37. Omar, S.A., 1998. the role of rock-phosphate-solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microb. Biot., 14: 211-218.
38. Rashmi, K.R., N. Earanna and M. Vasundhara, 2008. Influence of biofertilizers on growth, biomass and biochemical constituents of *Ocimum gratissimum* L. Biomed., 3(2): 123-130.
39. Singh, S. and K.K. Kapoor, 1998. Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions. Mycorrhiza., 7: 249-253.
40. Hornok, L., 1986. Effect of environmental factors on growth, yield and on active principles of some.
41. Demirezen, D., A. Aksoy and K. Uruc, 2006. Effect of population density on growth, biomass and nickel accumulation capacity of *Lemna gibba* (Lemnaceae) Chemosphere, 66: 553-557.

42. Atta, M.B., 2003. Some characteristics of (*Nigella sativa* L.) Seed cultivated in Egept and its lipid profile. Food Chemistry J., 83: 63-68.
43. Bhati, D.S., 1988. Effect of nitrogen application and row spacing on coriander (*Coriandrum sativum* L.) production under irrigated condition in semi arid Rajasthan. Indian Journal of Agriculture science, 58: 568-569.
44. Shalaby, A.S. and A.M. Razin, 1994. cultivation and fertilization for higher yield of thyme (*Thymus vulgaris*). Hort. Abs., 64: 1994.