Middle-East Journal of Scientific Research 8 (1): 01-09, 2011
ISSN 1990-9233

Effect of Mycorrhizal Symbiosis and *Bacillus coagolance* on Qualitative and Quantitative Traits of *Matricaria chamomilla* under Different Levels of Phosphorus

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Abstract: Currently, medicinal plants are of considerable interest in Iran. *Matricaria chamomilla* is amongst medicinal plants and regarding its wide distribution and essential oil components it has become a prominent plant. Medicinal plants have a superior performance and yield under organic farming conditions in comparison with conventional farming. A factorial design experiment was conducted on field to investigate the effects of Mycorrhizal fungi, (different species as one complex treatment) in two levels including non-inoculated (M0) and inoculated (M1) and Phosphate solubilizing bacteria *(Bacillus coagolance*) in two levels including non-inoculated (B0) and inoculated (B1) and Triple super phosphate in three levels including (P_0: 0 kg/ ha, P_1: 50 kg/ ha and P_2: 100 kg/ ha) on qualitative and quantitative traits of *Matricaria chamomilla.* Relevant characteristics including plant height, number of flowers per m^2, flower size, fresh and dry weight of flowers, dry weight of aerial parts as well as percentage and yield of essential oil and its components (kamazulens and Bisabololen compounds) were measured. Results revealed a significant effect of the interactive treatments in most studied properties and moreover enhancement in quality of yield was more than yield quantity. Establishment of an effective symbiotic relationship between this medicinal plant and arbuscular myccorrhizal fungi and efficient inoculation with phosphate solubilizing bacteria and application of triple super phosphate in proper amount (50 kg/ ha) resulted in augmentation in essential oil yield and its components (kamuzulen and bisabolen compounds).

Key words: *Matricaria chamomilla* · Phosphate solubilizing bacteria · Mycorrhizal fungi · Quantity and quality

INTRODUCTION

Matricaria chamomilla is one of the high consuming medicinal plants in Europe, Middle East, North and South of Africa, Australia and North America, which has been largely recognized because of its medicinal compounds. This plant is cultivated in order to make use of its beneficial blue essence in pharmaceutical and perfumery industry and preparing food flavors. This plant grows naturally in different regions of Iran (Khozestan, Khoramabad, Shiraz and Tehran provinces) and in some provinces intensive cultivation has been performed. Kamazulen is one of the major components of chamomile essence and consists of 5 percent of essence with anti-inflammatory, anti- allergy and anti- spasm effects. Bisabolol constitutes the main part of essence components with soothing, anti- bacterial and anti- fungal effects. Adequate nutrients contribution is one the most important strictures for plants in order to achieve higher yields [1, 2].

Within conventional agriculture the mentioned issue has been solved with application of chemical fertilizers [3]. In cultivation of medicinal plants, the real value is given to the quality while yield quantity comes in second step of importance. Some studies in concern of medicinal plants in natural and agro-ecosystems showed that sustainable agricultural approaches are the best methods in which these plants revealed better performance on the account of the harmony with nature, therefore global approach to medicinal plant production is leading toward

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sustainable agricultural systems [4]. In addition, environmental impacts which are caused by over application of chemical fertilizers, energies, expenses of their production and etc. are the reasons for global tendering toward application of bio-fertilizers [5]. Mycorrhizal fungi are beneficial microorganisms and hence, been considered as bio-fertilizer. Most terrestrial ecosystems depend on mycorrhiza, which promote the establishment, growth and health of plants. Improved productivity of AM plants was attributed to enhanced uptake of immobile nutrients such as Phosphorus, Zinc and Copper. Resistance against biotic and abiotic stresses has been argued to be due to the effects of AM fungi on inducing plant hormones production [6]. Phosphate solubilizing microorganisms are another sort of bio-fertilizers which have the ability to solubilize organic and inorganic phosphorus compounds by producing organic acid or phosphatase enzyme [7].

Many studies showed that these bacteria have a synergistic effect with mycorrhizal fungi and co-inoculation of them leads to more absorption of water and soil minerals and increases growth of host plant [8]. The effect of mycorrhizal symbiosis on 76 medicinal plants in Azad Jamma and Kashmir had been studied. Results showed different mycorrhizal root colonization. Plants at vegetative stage exhibited more VAM root colonization percentage compared to those at flowering and fruiting stages. Herbaceous plant showed more root colonization in comparison with shrubby and woody plants as well [9]. In two distinct researches which were carried out on lemon grass (*Symbopogon martini*) [10] and on mint (*Mentha arvensis*) [11], results showed that mycorrhizal inoculation caused increasing in percentage of essence and essence yield in comparison with non-inoculated. Their studies, revealed the improvement of nutrients in plants which caused enhancement of essence percentage and essence yield in inoculated plants. Kapoor *et al*. (2004) reported that fennel root symbiosis with two species of mycorrhizal fungi, including *Glomus macrocarpum* and *Glomus fasiculatum* significantly improved properties as follow; number of umbels in plant, seed weight, phosphorus concentration, biomass, percentage of AM root colonization,root and amount of essence (concentration of essential oil) [12]. Among two fungal species, *G.fasiculatum* showed the highest performance at both levels of phosphorus up to 78% increase in essential oil concentration of fennel seed over non- mycorrhizal control. Application of bio-fertilizers on chamomile showed the highest yield of fresh and dry weight of flowers and the highest essence yield obtained by application of phosphate solubilizing bacteria [13].

Darzi (2007) pronounced that application of bio-phosphate fertilizer (60 kg/ha) on fennel, had significant effect on essential oil concentration in seeds and essence yield [14]. In a research on lemon grass (*Symbopogon martini*) application of phosphate solubilizing bacteria with insoluble mineral phosphate ($Ca_3 (PO_4)_2$), illustrated that quality of essence increased and geraniol percentage in essence was about 27.6% more than control [8]. In the respect to the limited studies regarding the effects of bio-fertilizer applications on growth and yield of medicinal plant and particularly chamomile and respecting the importance of this plant for the production of essence, this research was conducted to investigate the effects of bio-fertilizers including; mycorrhizal fungi and phosphate solubilizing bacteria.

MATERIALS AND METHODS

This research was conducted in the research farm of Agriculture Faculty of Islamic Azad University, Karaj Branch- Iran with latitude of 35°48' and longitude of 50°57' and Altitude of 1235m. Soil samples were taken in depth of 0-30 cm in order to determine physical and chemical characteristics of soil via implementing common Lab methods.

In this Research 3 Factors Were Examined:

- Mycorrhizal fungi in two levels (M0: non- inoculated and M_1: inoculated).
- Triple super phosphate in three levels (P_0: 0kg/ha, P_1: 50kg/ha and P_2: 100kg/ha).
- Bio-phosphate in two levels: (B_0: non- inoculated and B_1: inoculated) with 10^8 (*Bacillus coagolance*) per ml.

The experiment was a factorial on the basis of Randomized Complete Block Design including 12 treatments and 4 replications.

Mycorrhizal inoculum had five species including; *Glomus etunicatum*, *Glomus caledonium*, *Glomus mossae*, *Glomus intraradices* and *Glomus sp* with equal spore population. These species reproduced by corn pot culture within 4 months in sterilized sand media in soil and water research institute. Every each of *chamomile* seeds was inoculated by 200-250 active fungal propagules by mixing mycorrhizal inoculum with chamomile seeds. Both two of bio-fertilizers prepared in soil and water research institute in Tehran. Each experimental plot was 3×2.4 m^2 and contained 5 lines of planting, with 1m distance

between two rows and distance between each blocks was 2 m. Planting of chamomile and applying experimental treatments were executed in accordant conditions of spring weather in 2010. In 4- lateral- branch stage, plantation density adjusted base on 40cm×10cm and distance between plants were 10cm. Bio-phosphate was added as bacterial solution in plant establishment and root development stages. 30 kg/ ha of nitrogen fertilizer (Urea) was applied in top dressing form when plant height reach to 7-9cm. In this research properties screened as follow: Number of flower per plant, diameter ratio and size of flowers, fresh weight of flowers, dry weight of flowers, dry weight of above-ground organs, essence percentage, essence yield, quality of essence, percentage of root colonization with mycorrhizal fungi in each plot and from middle line . Following drying wet flowers in fresh air and shadow, 100 g of dry flowers from each plot collected and prepared for measuring essential oils. Afterwards the sampling of plants deducted from selected area and put in oven for two days and then dry weight and dry weight of flowers were measured. Flowers diameter and plant's height were measured as well.

Extraction of essence and identity of its compounds. From each experimental plot, 100-grams of dried flowers were prepared and essence acquired after 3 hours by water distillation and Clevenger instrument. Yield of essence was calculated after dehydrating of water by sodium Sulfate [12]. GC- mass was utilized for essence analyzing and exact measurement of its ingredients according to Azulen and Bisabolols was defined. One mixed sample selected from repetition of each treatment and was analyzed for determination of its ingredient.

Determination of root symbiosis with mycorrhizal fungi: Cleaning and staining of root samples performed in accordance with Philips and Hayman method (by the use of KOH 6% in 4 hours for bleaching and solution of 5% trypan blue in lactoglycirol for staining) [16]. Mycorrhizal symbiosis percentage was measured by Gridline intersect method of Norris, *et al*. 1994.

RESULTS AND DISCUSSION

Number of Flowers: Results revealed that inoculated mycorrhizal treatments in 5% level of significancy had effect on number of flowers and through application of mycorrhizal inoculation, number of flowers increased about 6.5%. Another research on fennel showed that number of umbrella in plant with mycorrhizal inoculums was increased 17.5% [14]. Also a two year research on tomato revealed that symbiosis of tomato's root with- on species of mycorrhizal fungi led to great enhancement in number of flowers per plant [17].

Plant Dry Weight: Mycorrhizal inoculation, bio-phosphate and TSP hadn't significant effect on plant dry weight but interaction of TSP and bio-phosphate and also interaction of TSP and mycorrhizal inoculation were meaningful ($P < 0.01$) and interaction of bio-phosphate and mycorrhizal inoculation was also meaningful ($p < 0.05$) on improvement of dry weight. Another research on wheat exhibited that mycorrhizal plants in comparison with non- mycorrhizal plant had more dry material of stem and root and root length [18]. Interactive effect of bio-phosphate and TSP, at second level of phosphorus (50 kg/ ha) in presence of bio-phosphate had the highest effect on dry material of while in the third level of TSP (100 kg/ ha) with non- inoculated treatments of bio-phosphate exhibited the lowest amount of plants dry weight. The highest amount of dry weight in second level of TSP and mycorrhizal inoculation and lowest dry weight of plant in

Table 1: Analysis of variances for measured traits

		Mean Squares (MS)							
SOV	df	Flower number	Flower diameter	Flower dry weight	Flower fresh weight	Bush dry weight	Essence %	Essence yield	Colonization %
Mycorrhiza	1	*	*	*	*	ns	ns	ns	**
Biophosphate	1	ns	ns	ns	ns	ns	ns	ns	ns
Mycorrhiza×Biophosphate	1	ns	ns	ns	ns	ns	*	*	ns
TSP	2	ns	ns	ns	ns	ns	ns	ns	*
Mycorrhiza×TSP	2	ns	ns	ns	ns	*	ns	**	ns
Biophosphate×TSP	2	ns	ns	*	*	**	ns	**	ns
Tree-fold interaction	1	ns	ns	ns	ns	ns	ns	ns	ns
Error	37	1014034.02	0.7434	34459.46	1358822.09	13830.35	0.0077	13830.31	83.797

ns, non-significant; **, significant at p = 0.01; *, significant at p = 0.05; TSP, triple super phosphate

third level of TSP and non- mycorrhizal inoculation has been appeared. Interaction between three factors showed the highest amount of dry weight which were obtained with inoculation treatments and second level of TSP and in these treatments bio-phosphate hadn't any effective results. Another research on orange seed showed that inoculation of orange seed with mycorrhizal fungi (*Glomus fasiculatum*) enhanced growth and resulted in stronger transplants over control. Average dry weight of inoculated transplants was reported more than control After 24 weeks [19].

Average of Flower Diameter: Mycorrhizal inoculation was the only significant factor on average of flower diameter ($p < 0.05$) with negative- effect on this parameter. Considering the effect of mycorrhizal inoculation on increasing number of flower, decreasing of flower diameter is seems to be reasonable. Since the active essential oil producing organs in chamomile are yellow and shiny and cone capital, therefore decrease in flowers diameter hadn't any meaningful effect on percentage of essence compound.

Fresh Weight of Flower Yield: Mycorrhizal inoculation had significant effect ($p < 0.05$) on fresh weight of flower, but other main treatments including bio-phosphate and TSP hadn't significant effects on fresh weight of flower and this results indicated that the plant had more compatibility with mycorrhiza bio-fertilizer. In mycorrhizal inoculation treatments, regarding the higher absorption of water and mineral elements, especially elements with low mobility in soil such as phosphorus, Fe and Zn, fresh weight of flowers had significantly enhanced [20]. In treatments with mycorrhiza inoculation, fresh weight of flower was 8% higher than non- inoculated treatments.

In a nearly corresponding research on corn, application of mycorrhizal inoculum increased the yield of fresh forage from 49 ton to 52.3 ton per hectare [21]. Interaction of TSP and bio-phosphate had significant effect on increasing fresh weight of flowers. In case of properties like plant height, the best bio-fertilizer treatment was the use of bio-phosphate and second level of TSP. other interactions resulted in no significant effect.

Dry Weight of Flower Yield: Mycorrhizal inoculation had significant effect ($p < 0.05$) on enhancement of dry weight of flower, but other main factors hadn't significant effect. Interaction between bio-phosphate and TSP was substantially significant ($p < 0.05$). Bio-phosphate with second level of TSP had better effect rather than other treatments on dry weight of flower. A research on bean illustrated that production of dry material, amount of photosynthetic pigments, relative water content, phosphatase activity were significantly higher in inoculated plants in comparison with non- mycorrhizal plant, but this advantageous effect decreased with increasing of phosphorus content of soil [11].

Value of Essence or Percentage of Essence: None of three factors had significant effects on essential oil production.

Interaction of bio-phosphate and mycorrhizal fungi had significant effect ($p < 0.05$) on essential oil production and the highest percentage of essence produced by simultaneous application of mycorrhizal inoculation and bio-phosphate. Essential oils are Terpenoid compounds and their productive units are isoprenoids such as Isopentenyl pyrophosphate (IIP) and Dimethyl allyl pyrophosphate (DMAPP) which their production needs ATP and NADPH, thus for this formation, N and P are essential [14]. Deliberating the mutualistic symbiosis, application of bio-phosphate and enhancement in P and N and other elements with low mobility in soil (Fe, Zn, Cu) by roots resulted in augmentation in essential oil compounds.

Results of an experiment on lemon grass (*Symbopogon martini*) showed that application of mycorrhzial fungi and phosphate solubilizing bacteria in presence of phosphate rock led to enhancement in activity of both microorganisms and fortification and intensification of essence in this medicinal plant [8]. This research unveiled that bio-fertilizer treatments can provide a better condition for improvement of beneficial microbial activity in soil. Although other factors hadn't any significant effect, but mycorrhizal inoculation caused the enhancement in value of essence. In relation to interaction of phosphorus and mycorrhizal fungi the highest percentage of essential oil belonged to second level of TSP with mycorrhizal inoculation and the lowest percentage of essence relates to third level of TSP without mycorrhizal inoculation.

The highest percentage of essential oil observed in second level of TSP, inoculation with mycorrhizal fungi and bio-phosphate while the lowest percentage of essence remarked in third level of TSP and without application of bio-fertilizers.

Essence Yield: Interaction of TSP with bio-phosphate and TSP with mycorrhizal inoculation at ($p < 0.05$) and also interaction of bio-phosphate and mycorrhizal inoculation

($p < 0.05$) had meaningful effects. Second level of TSP (50 kg/ ha) with bio-phosphate had the highest essence yield and third level of TSP without application of bio-phosphate had the lowest essence yield. The highest essence yield obtained with application of (50 kg/ ha) TSP and application of bio-phosphate. Thus lack of phosphorus or use of high level of it led to the lowest essence yield. In a research on fennel, mycorrhiza application and bio-phosphate had meaningful effects ($p<0.01$) on essence yield [14]. Gupta *et al*. (2002) reported that colonization of mint root with mycorrhizal fungi caused an eminent of enhancement in essence yield [22].

Percentage of Root Colonization: Mycorrhizal inoculation ($p<0/01$) and TSP ($p<0/05$) had significant effects on root colonization in chamomile plant. Average comparisons elucidate that percentage of root colonization in mycorrhizal inoculation treatments had a superiority effect over non- inoculated control. Whilst colonization percentage at third level of TSP (100 kg/ ha) was 45% less than first level and in second level (50 kg/ ha) was less than first level around 28%.

Kapoor *et al*. (2004) reported that root colonization percentage in fennel in inoculated treatments with two species of mycorrhizal fungi (*Glomus fasiculatum*, *Glomus macrocarpum*) was substantially more than non- inoculated treatments [12]. Researchers announced that inoculation of mycorrhizal fungi (*Glomus fasiculatum*) with mint root caused great increase in percentage of colonization (287%) [221]. Also in natural researches on *Rosmarinus officinalis* and on *Lavandula Spica* the same results were obtained [24-26].

Value of Azulen in Essence: Results demonstrated that both mycorrhizal inoculation and phosphate solabilizing bacteria were effective on value of Azulen in essence. Azulen percentage of essence with mycorrhizal inoculation in comparison with non- inoculated treatments revealed about 3.1% of enhancement. High value of Azulen in chamomile's essence acts to demonstrate the high quality of essence of this medicinal plant and a decent mycorrhizal symbiosis which improves uptake of nutritional elements and an enhancement of essence azulen percentage remarked by chamomile. A research concerning the effects of mycorrhizal association on the concentration and composition of essential oil in coriander showed that VAM inoculation increased the concentration of the essential oil in fruits up to 43% and caused improvement of essence quality, therefore the amount of consequential components like Geranial (19.99%) linalool (61.73%) significantly increased in mycorrhizal treatments in comparison with non-inoculated control [27].

Results from comparable researches approved the indicated effect of mycorrhizal symbiosis on quality improvement of medicinal plant essence. Ratti *et al*. (2001) reported an increasing in Geraniol of plamarosa and Kapoor *et al*. (2002a) reported an increasing in beta-farnesene, Persian and Alfa morphine in carum [28, 29]. Researchers showed that inoculation of two vesicular arbuscular mycorrhizal fungi including *Glomus clarum* and *G. margarita* led to improvement of essence quality in comparison with non- inoculated control [29].

So that menthol content in essence has increased about 89% in comparison with control. It's been apprised that the significant enhancement of essence quality as a consequence of betterment in nutrients uptake happened due to mycorrhizal symbiosis while application of phosphorous alone resulted in no substantial effect on improving menthol content in mint essence.

Table 2: Physical and chemical properties of soil

C (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	K (mg/kg)	P (mg/kg)	N (mg/kg)	O.C (%)	Ec (ds/m)	pH	Texture
0.1	7.68	3.66	16.33	452	0.7	1.5	1.1	1.79	7.7	Loam

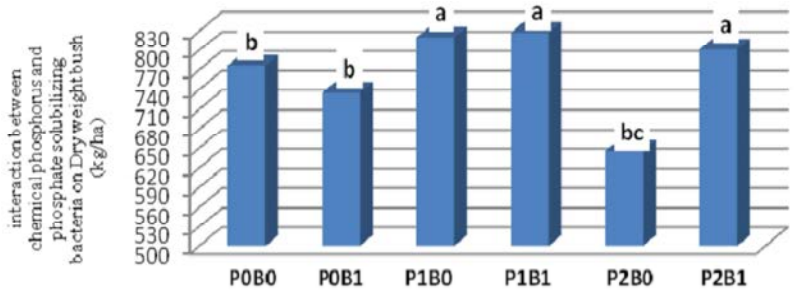

Fig. 1: Mean Comparison for interaction effect of chemical phosphorus and phosphate solubilizing bacteria on Dry weight bush.

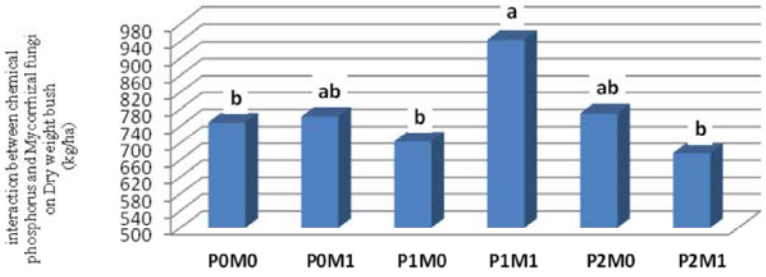

Fig. 2: Mean Comparison for interaction effect of chemical phosphorus and Mycorrhizal fungi on Dry weight.

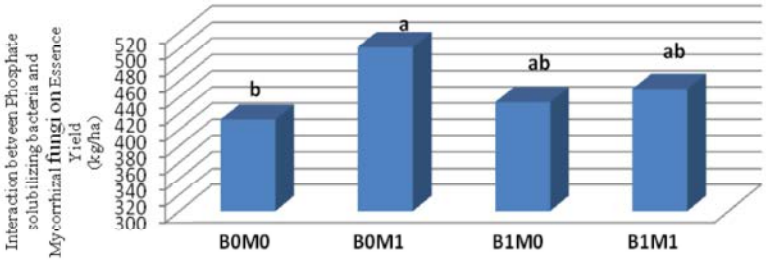

Fig. 3: Mean Comparison for interaction effect of Phosphate solubilizing bacteria and Mycorrhizal fungi on Essence Yield.

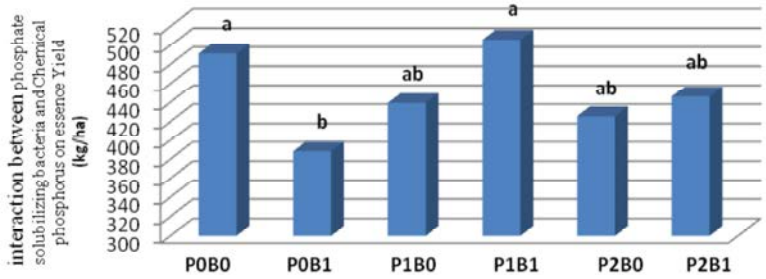

Fig. 4: Mean Comparison for interaction effect of Phosphate solubilizing bacteria and Chemical phosphorus on Essence Yield.

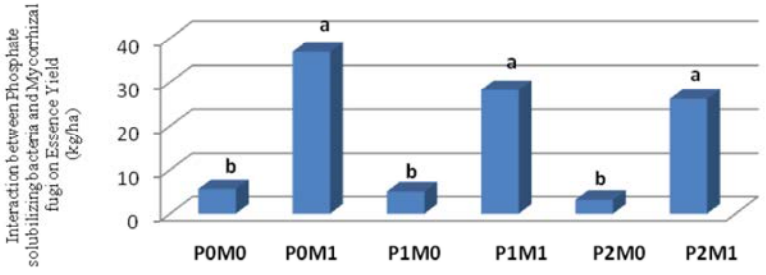

Fig. 5: Mean Comparison for interaction effect of Phosphate solubilizing bacteria and Mycorrhizal fungi on Essence Yield.

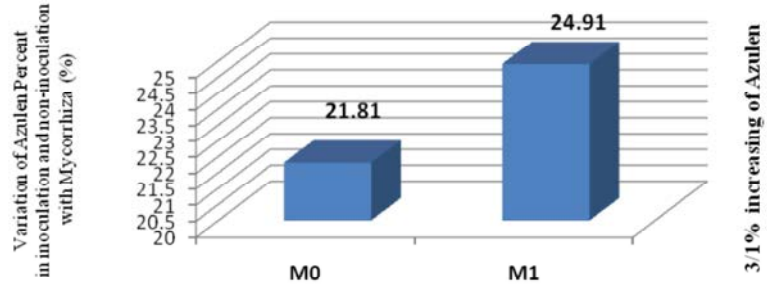

Fig. 6: Azulen percent in inoculated and non-inoculated with Mycorrhizal fungi.

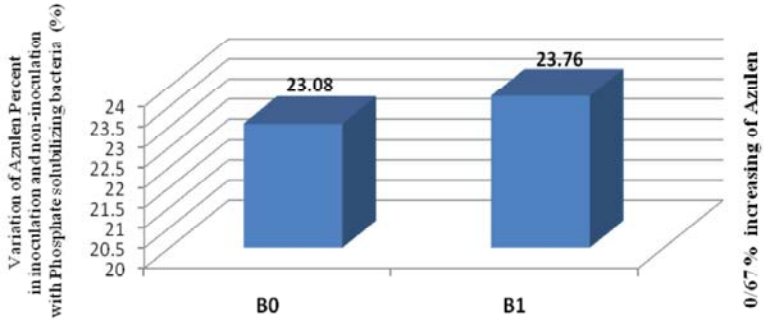

Fig. 7: Azulen percent in inoculated and non- inoculated with Phosphate solubilizing bacteria.

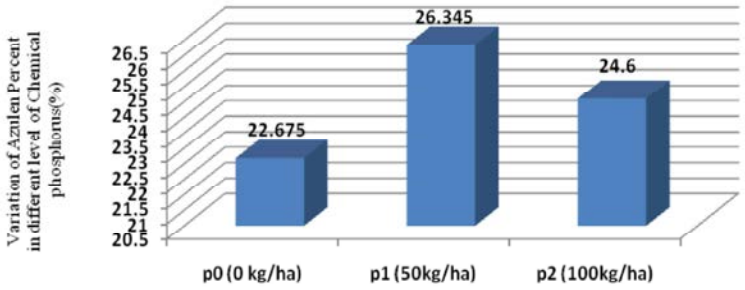

Fig. 8: Azulen percent in diffrent level of Chemical phosphorus.

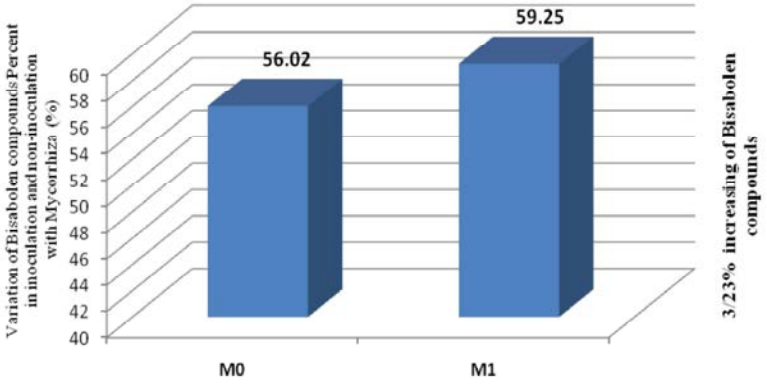

Fig. 9: Bisabolen compounds percent in inoculated and non- inoculated with Mycorrhizal fungi.

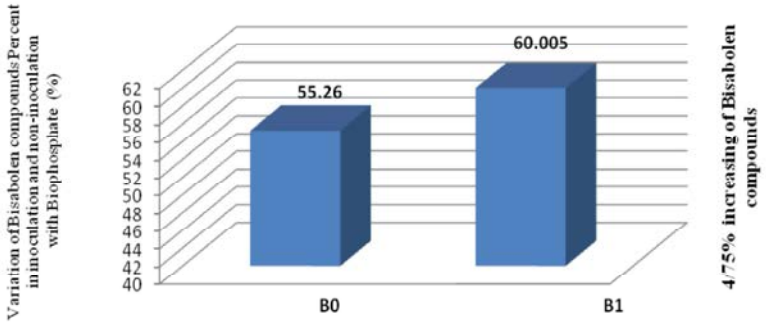

Fig. 10: Variation of Bisabolen compounds percent in inoculated and non- inoculated with Biophosphate.

Quality of essence depends exceedingly on symbiotic fungi and plant's species and regarding such circumstances researchers reported different results from different treatments.

In another research concerning the effects of phosphate solubilizing bacteria on essence quality of Palmarosa [8] it has been showed that Geraniol in essence was 27.6% higher relative to non- inoculated control.

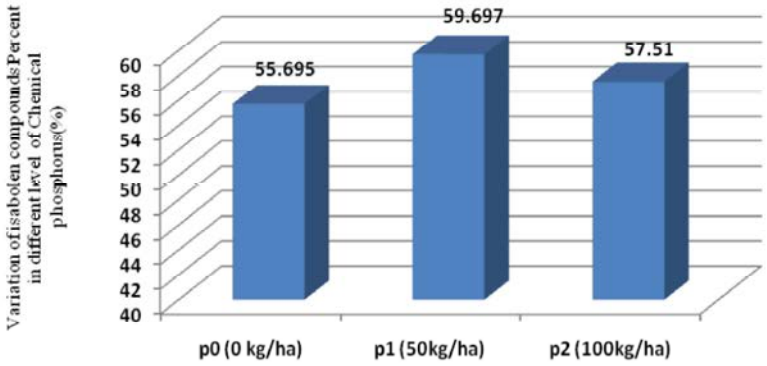

Fig. 11: Variation of Bisabolen compounds percent in different level of Chemical phosphorus

Results of a study on sugar cane approved this result [30]. Likewise different treatment comparison of TSP on Azulen content showed that value of Azulen in second level of TSP (50 kg/ ha) was the highest content (26.345) which in comparison with control or first level of phosphorus revealed 16.62% of enhancement, but there was 6.86% decrease in Azulen content in third level of phosphorus (100 kg /ha) in comparison with first level of phosphorus (0 kg/ ha). It declares that second level of phosphorus (50 kg/ /ha) had the highest content of Azulen (26.345).

Bisabolen's Composition in Essence: Results illustrated that both of mycorrhizal fungi and bio-phosphate were effective on bisabolen's composition, in essence and relative to non- inoculated treatments.

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