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The effects of biofertilizers and different amount of chemical fertilizers on some growth traits and essence yield of chamomile (*Matricaria recutita* L.)

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Abstract

To evaluate the effect of bio-fertilizers and different levels of recommended chemical fertilizers on some of growth traits and essence yield of chamomile (*Matricaria recutita* L.), the experiment was performed in form of split plot based on randomized complete block design with three replications on May 2013 at lands of forest park in Tabriz Shahid Ghazi pharmacy located at 20km of Tabriz-Tehran road. The main factor was involved chemical fertilizers at three levels of no consumption, 50% and 100% consumption of recommended amount and Subplot factor had six levels of bio-fertilizers which were included: control, Nitrajin, Phosphatin, Thiyobacillus, Biosuper and cow rotten manure. The results of variance analysis demonstrated that application of different biological fertilizers, chemical fertilizers and their combination had significant effect on majority of studied traits at 5% probability level. Nitrajin bio-fertilizer without chemical fertilizers had the greatest effectiveness due to high influence on traits such as essence yield. Bio-fertilizers of Thiyobacillus and Biosuper both with 50% of recommended chemical fertilizers and also manure with 100% of chemical fertilizer, respectively were in next categories and had no significant differences with treatment of just Nitrajin. The results of this study represented that with consideration of importance in non-use of chemical fertilizers in agricultural production especially medicinal plants; for production of chamomile, usage of Nitrajin bio-fertilizer without chemical fertilizers was better treatment than other combinations.

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Introduction

Diversity and dispersion of plant species in plains and mountains of Iran are about twice species that exist in several European countries, for this reason some experts in the world know Iran as repository of medicinal and aromatic plants (Omidbeygi, 2006). German chamomile with scientific name of *Matricaria recutita* L. from *Asteraceae* family is one of the most important medicinal plants which is cultivated in the world especially in Europe and uses a lot in pharmaceuticals, cosmetics, health and food industry (Letchamo, 1992; Pirkhezri *et al*., 2008).

Annual consumption of chamomile in the world is about more than 4000 tons of dried flowers, which most of it was involved German chamomile (Fallahi *et al*., 2009). In recent years, numerous researches have been done about use of chemical fertilizers, particularly nitrogenous and phosphorous fertilizers on crops, but more researches need to be done about consumption of these fertilizers and their management on secondary metabolites in medicinal plants such as chamomile (Lopes, 1996). Use ease and also low cost of chemical fertilizers have been led to bio-fertilizers don't use more, which caused crisis of environmental pollutions, especially pollution of soil and water resources (Sing and Kapoor, 1998).

Bio-fertilizers contain preservatives with high density of one or more types of beneficial soil micro-organisms or in form of metabolites products which form colony around plant roots or inside parts of plants, and stimulate growth of host plants with various ways (Rahmani *et al*., 2008). Nowadays, with consideration of special privileges in bio-fertilizers including economic benefits, reduce environmental pollution, reduce production costs and improve quality; usage of these fertilizers especially in the field of drug plants production has become more important (Kandeel *et al*., 2002). Beneficial soil micro-organisms which are called PGPR have ability in improvement of plant growth by providing plant nutrients, secretion of growth hormones and organic acids that caused to enhancement of soil fertility and protect health of environment (Esitken *et al*., 2010).

Records from previous decades, show that the application of chemical element on farm lands caused some environmental problems such as water pollution, low quality of agricultural productions and decrease in soil productivity rate (Sharma, 2002). Sustainable farming on the base of natural fertilizer application with the aim of omitting or decreasing chemical elements is a desirable approache to solve these difficulties. Natural fertilizers are both economically desirable and stable soil sources, in maintaining long time production and prevention of environmental pollution (Saleh, 2001).

Anna *et al*., (2004) confirmed that by the application of phosphate solvent bacteries, a significant yield improvement was obtained in (*Phyllanthus amarus*). Wasule *et al.,* (2002) mentioned that phosphate solvent bacteries and *Bradyrhizobium japonicum* application on soybeen increased significantly improved some characteristics such as nodulation, dry weight of nodules and plant dry weight.

It should be noted that most parts of Iran have poor soils and low organic matter, so the importance of this research in this field is clear. Accordingly, this study aimed to evaluate the effects of bio fertilizer and amount of chemical fertilizers on the growth traits and essence yield of chamomile (*Matricaria recutita* L.).

Materials and methods

Plant material and growing conditions

Experimental design was carried out in form of split-plot based on randomized complete block with two factors and three replications. Two factors were involved: main factor were: non-use of chemical fertilizer, 50% consumption of recommended chemical fertilizer and 100% recommended chemical fertilizers. Subplot factor with 6 levels were bio-fertilizer: non-use of fertilizer, manure, Nitrajin, Phosphatin, Thiyobacillus and Biosuper. Used fertilizers were contained nitrogen, phosphorus and potassium, respectively from resources of urea, triple superphosphate and potassium sulphate which were used in form of mixture with field soil based on

recommended fertilizer from soil analysis test.

Biofertilizers and chemical fertilizers application

In this experiment, fertilizers of Nitrajin, Phosphatin and Biosuper were consumed as rubbed seed, but Thiyobacillus should be mixed with sulfur and rotten manure was used in form of consumption soil. Chamomile seeds mixed with different organic fertilizers and also pure seed in treatment of control, manure and Thiyobacillus were planted in rows. Each main plot consisted of three different types of fertilizers which were contained non-use of chemical fertilizer, 50% and 100% of recommended fertilizer.

Planting conditions

There were six sub-plots in each main plot which had levels of factor B. Thus, there were 18 sub-plots in each replication and with numeration of three replication, 54 plots were available in total. In this research, field preparation including: plowing was carried out to create soft soil to prepare seed bed thoroughly. In sowing operations, for prevention from wind and movement by water and steady spatter, seeds washed with some tiny and soft sand, then mixed and were planted uniformly in rows. Plots were based on research design in the field which had dimensions of 12×41m and area of 500 square meters approximately. Each plot had 5 rows. Length of each planting row was 2.5 meters and spacing between rows within each treatment was 25 cm. Distance between each treatment and distance between main plots from each other were 0.5 m and one meter, respectively. Based on calculations and fertilizers recommendations according to soil test in laboratory; 1.5kg of urea fertilizer, 250g potassium sulfate and 250g of triple super phosphate were mixed for treatments of 50% chemical fertilizer per 100 square meters and were added to field soil in considered locations. In treatments of 100% chemical fertilizer, theses amount were included: 3kg of urea, 500 g. of potassium sulfate and also 500 g. of triple superphosphate were mixed with soil per 100 square meters of field soil in considered locations. Base of manure consumption was 30 tones per hectare which were used in considered rows. Manure was cow type which maintained during two years in special pit and operation of maceration and converse on it was done to eliminate weeds and also performance of chemical interactions. After emergence, plants spacing reached to 10cm by thinning. Irrigation was done according to plant needs and weather conditions. In this experiment, irrigation was done once a week except initial irrigation after planting which had been carried out with carrousel to avoid seed washing and an extra irrigation on August due to weather extreme heat. No kind of herbicide for removing weeds was used because of drug product. Hand weeding was done in several occasions during growing season. Three consecutive moving harvested due to favorable weather conditions during growing season and traits measurement were performed on them.

Statistical analysis

Variance analysis of data was done after controlling for normality of data distribution and Duncan's multiple range test at 5% probability level was used for mean comparisons. Statistical calculations included analysis of variance and mean comparison was done with use of MSTAT-C software.

Results and discussion

Variance analysis of experimental factors effects on studied traits have been shown in Table 1.

Plant height

First cutting

The highest plant height with amount of 46.34cm was obtained in treatment of non-use of bio-fertilizer with 50% recommended consumption of chemical fertilizer which had 29.78% increase and significant difference as compared with control treatment. The lowest plant height with amount of 31.38cm was related to treatment of 100% recommended chemical fertilizer which had 3.56% decrease as compared with control treatment. In total of treatments, maximum plant height was achieved in treatment of 50% consumption of chemical fertilizer with non-use of bio-fertilizer which demonstrated 29.78% augmentation as compared with control treatment and minimum plant height was obtained in treatment

of 100% consumption of chemical fertilizer with non-use of any bio-fertilizer which represented 3.56% decrement as compared with control treatment (Fig. 1).

Second cutting

The highest value was equal to 35.35cm, which was obtained from control treatment without any fertilizer. 50% consume of recommended chemical fertilizer reached plant height to 33.73cm. Among three levels, chemical fertilizer had the lowest amount and revealed 4.58% decrease in comparison with control treatment. In 100% consumption of recommended chemical fertilizer, plant height was 34.36cm which decrease was less about 2.8% and had no significant difference with control treatment (Fig. 2). Research results of Youssef *et al.,* (2004) indicated that in drug plant of Salvia (*Salvia officinalis* L.), use of bio-fertilizer containing Azotobacter and Azospirillum, caused to augmentation in plant height, dry and fresh weight of shoot plants in first and second moving during two seasons.

Table 1. Analysis of variance for studied traits in chamomile under use of chemical and biological fertilizers.

S.O.V.	df	M.S.											
		Plant height			Branch number			Flower number			Essence yield		
		First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting
Replication	2	15.61	17.32*	1.23	12.87	4.74	2.83	15.68	3.25	40.89	0.15	0.17	3.90
Chemical fertilizer A	2	62.16ns	12.1*	10.61ns	153.6*	113.7*	66.7*	125.6**	84.3ns	46.5ns	0.37ns	5.49ns	15.63ns
Error 1	4	11.9	1.25	8.73	8.69	10.5	4.82	4.87	16.34	23.33	1.79	1.91	1.58
Biological fertilizer B	5	30.03*	11.05ns	14.3ns	38.3**	24.02ns	4.68ns	64.17**	31.9ns	43.77*	2.83*	5.57**	3.29*
A×B	10	53.16**	26.5ns	11.09ns	31.26**	63.3**	53.3**	88.7**	21.6ns	117.6**	2.01*	2.78**	3.40*
Error 2	30	11.26	12.49	9.72	6.02	9.78	8.9	10.26	15.49	13.64	0.86	0.64	1.29
C.V (%)		8.77	10.25	8.86	15.41	18.93	16.97	21.01	23.22	20.24	27.46	16.2	15.51

ns: non significant, * and ** significant at 5% &1% respectively.

The number of branch

First cutting

Maximum number of branch per plant with an average of 25.73 stems were obtained in treatment of non-use of bio-fertilizer with 50% consumption of chemical fertilizer that indicated 33.15% increase in comparison with control treatment and minimum number of stems per plant with value of 13.3 was related to treatment of 100% recommended chemical fertilizer which demonstrated 22.67% reduction even as compared with control treatment. In total of treatments, maximum number of branches per plant was achieved in first cutting and at treatment of 50% consumption of chemical fertilizer with non-use of bio-fertilizer and also in treatment of Nitrajin with 50% consumption of recommended chemical fertilizer with amounts of 25.73 and 24.87 stems, respectively; which represented 33.15% and 30.84% increase, respectively as compared with control treatment. The lowest number of branches per plant (9.93) was obtained in treatment of Phosphatin bio-fertilizer without any chemical fertilizer which reveals 42.25% reduction as compared with control treatment (Fig. 3).

Second cutting

In treatments with non-use of bio-fertilizer and only levels of recommended chemical fertilizers; maximum number of branches per plant was obtained in 100% consumption of chemical fertilizer with average number of 20.33 and 21.3% augmentation as compared with control treatment. The lowest number with value of 13.8 was related to 50% of recommended chemical fertilizer that, displayed

13.75% reduction in comparison with control treatment. In usage of Biosuper as a bio-fertilizer with different levels of recommended chemical fertilizers, maximum number of branches with amount of 16.53 was achieved in application of Biosuper with 100% of chemical fertilizers and had increment about 3.21% as compared with control treatment. The lowest number of branches per plant was 11.13 which was obtained in application of Biosuper with 50% of chemical fertilizer and had decrement about 30.44% as compared with control treatment. Overall, among 18 treatments combinations; the largest number of branches per plant with rate of 25.13 was related to second cutting with usage of Thiyobacillus fertilizer and 100% of recommended chemical fertilizer which had 36.33% augmentation in comparison with control treatment. The least number of branches per plant with amount of 8.6 was belonged to treatment of Nitrajin without use of recommended chemical fertilizers which demonstrated 46.25% decrease as compared with control treatment (Fig. 4).

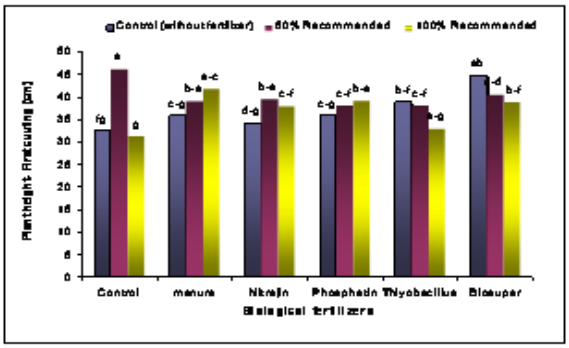

Fig. 1. The effect of biological fertilizers and amount of chemical fertilizers on plant height in first cutting.

Third cutting

In treatments with non-use of bio-fertilizer and only levels of recommended chemical fertilizers; maximum number of branches per plant was obtained in 50% consumption of chemical fertilizer with average number of 24.13 and also 42.27% augmentation as compared with control treatment. The lowest number with value of 13.93 was related to control treatment without any fertilizers. No significant difference was found in treatments with manure and consume of different levels of chemical fertilizers. The largest number of stems per plant was achieved in use of manure with no chemical fertilizer which was equivalent to 18.93 stems and had 26.41% increment as compared with control treatment. The least number of stems per plant with amount of 16.47 was related to usage of manure with 50% of recommended chemical fertilizer and also had 15.42% augmentation as compared with control treatment. Best results were obtained in consumption of Nitrajin with chemical fertilizers in comparison with usage of Nitrajin without chemical fertilizers. Overall, among treatments combinations; maximum number of branches per plant with rate of 24.13 was related to third cutting with usage of Thiyobacillus and 50% of chemical fertilizer and without bio-fertilizers which had 42.27% augmentation in comparison with control treatment. The least number of branches per plant with amount of 10.67 was belonged to treatment of Biosuper without use of recommended chemical fertilizers which demonstrated 23.4% decrease as compared with control treatment (Fig. 5). Letchamo (1992) assessed effects of ammonium nitrate fertilizer (NH_4NO_3) on qualitative and quantitative properties of German chamomile in pots containing 0 saplings. In this study; plant height, number of main and secondary branches, flowering rate, essence amount and flavonoids percentage reached to maximum amounts in half a gram of fertilizer level per pot.

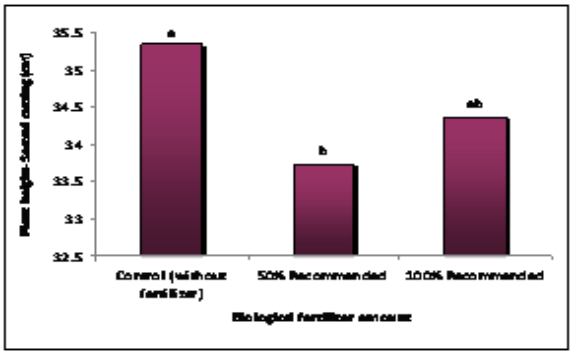

Fig. 2. The effect of biological fertilizer on plant height in second cutting.

Number of flowers

First cutting

Use of chemical fertilizers in terms of non-use of biofertilizers had significant alterations toward each others and number of flowers per plant increased with augmentation of fertilizer amount. Among these treatments, maximum number of flowers per plant with rate of 21.7 was related to treatment of 100%

chemical fertilizers which had 60.37% augmentation in comparison with control treatment. In first cutting, minimum number of flowers per plant with amount of 8.6 was obtained in control treatment without chemical fertilizers. In treatments with use of manure and different levels of recommended chemical fertilizers; maximum number of flowers per plant with amount of 23.33, was achieved in use of manure with 50% chemical fertilizers which had 63.14% increment as compared with control treatment. The least number of flowers per plant with value of 11.87 was related to treatment of manure without recommended chemical fertilizers which had 27.55% augmentation in comparison to control treatment.

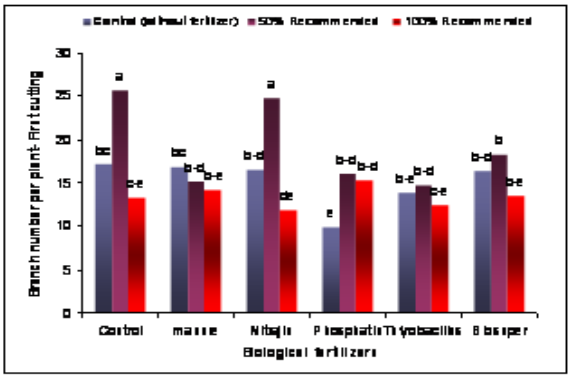

Fig. 3. The effect of biological fertilizers and amount of chemical fertilizers on branches number in first cutting.

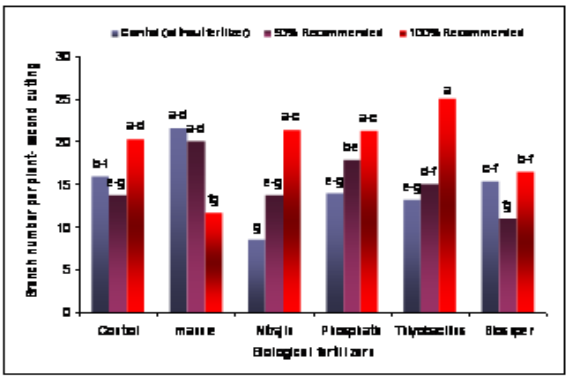

Fig. 4. The effect of biological fertilizers and amount of chemical fertilizers on branches number in second cutting.

Overall, among different treatments combinations; the highest number of flowers per plant with rate of 23.33 was related to first cutting with usage of manure with 50% chemical fertilizers which had 63.14% increase as compared with control treatment. The least number of flowers per plant with amount of 7.2 was belonged to treatment of Thiyobacillus with 100% of recommended chemical fertilizers which had 16.28% decreases in comparison to control treatment (Fig. 6).

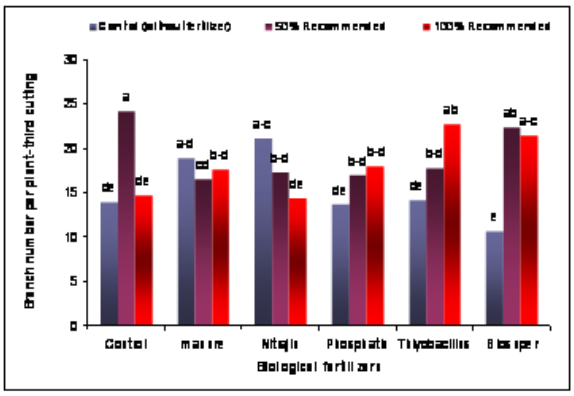

Fig. 5. The effect of biological fertilizers and amount of chemical fertilizers on branches number in third cutting.

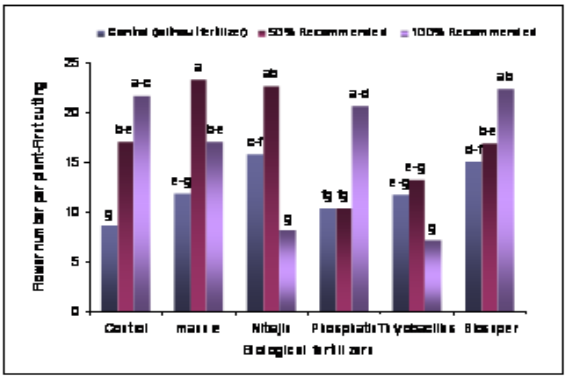

Fig. 6. The effect of biological fertilizers and amount of chemical fertilizers on flowers number in first cutting.

Third cutting

Consume of chemical fertilizers in terms of non-use of bio-fertilizers had significant alterations toward each others. Among these treatments, maximum number of flowers per plant with rate of 24.37 was related to treatment of 50% chemical fertilizers which had 28.31% augmentation in comparison with control treatment. Minimum number of flowers per plant with amount of 12.87 was obtained in usage of 100% chemical fertilizers and represented 26.33% decrement as compared with control treatment. In treatments with use of manure and different levels of recommended chemical fertilizers; maximum number of flowers per plant with amount of 27.35, was

achieved in use of manure with 100% chemical fertilizers which had 36.54% augmentation in comparison to control treatment. The lowest number of flowers per plant with value of 15.2 was related to treatment of manure with 50% of recommended chemical fertilizers which had 12.99% reduction as compared with control treatment.

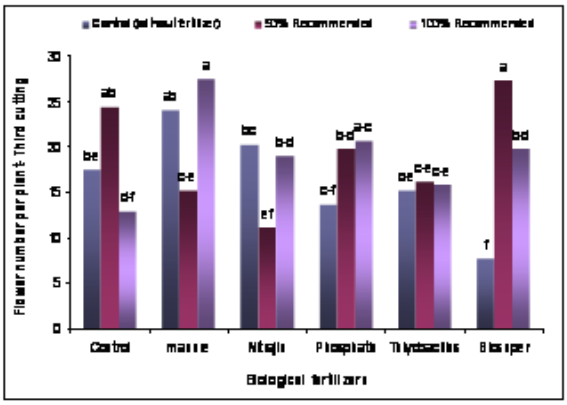

Fig. 7. The effect of biological fertilizers and amount of chemical fertilizers on flowers number in third cutting.

Overall among different treatments combinations in third cutting, the highest number of flowers per plant with rate of 27.53 was related to usage of manure and 100% chemical fertilizers and also had insignificant difference with treatment of Biosuper and 50% chemical fertilizer with amount of 27.4 which had 36.54% and 36.24% increase, respectively as compared with control treatment. Minimum number of flowers per plant with amount of 7.75 was belonged to treatment of Biosuper without recommended chemical fertilizers which had 55.65% reduction in comparison to control treatment (Fig. 7).

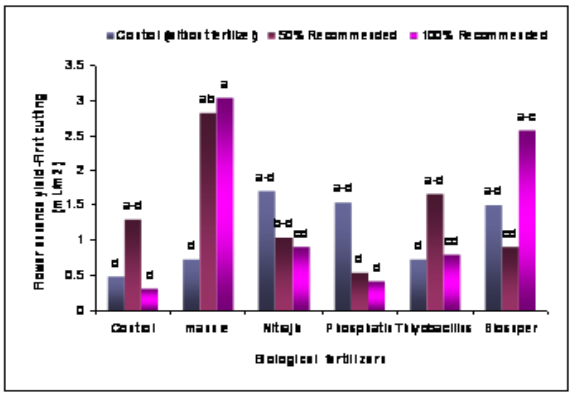

Fig. 8. The effect of biological fertilizers and amount of chemical fertilizers on flowers essence yield in first cutting.

Hernandez *et al.*, (1995) declared chemical fertilizer is effective in augmentation of flower production in drug plant of chamomile, due to availability of necessary nutrient elements for plant growth.

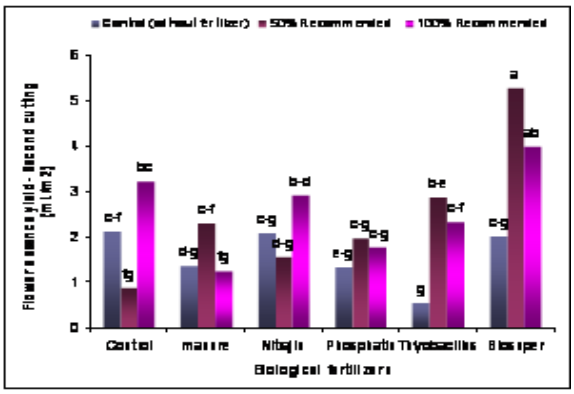

Fig. 9. The effect of biological fertilizers and amount of chemical fertilizers on flowers essence yield in second cutting.

Yield of flower essence

First cutting

Usage of chemical fertilizers in terms of non-use of bio-fertilizers had significant alterations toward each others. Among these treatments, the highest essence yield with rate of 1.307 mL/m² was related to treatment of 50% chemical fertilizers which had 62.81% augmentation in comparison with control treatment. The lowest essence yield with amount of 0.316 mL/m² was obtained in usage of 100% chemical fertilizers and had 34.98% decrement as compared with control treatment. In treatments with use of manure and different levels of chemical fertilizers; maximum yield of essence with amount of 3.043 mL/m², was achieved in use of manure with 100% recommended chemical fertilizers which had 84.03% augmentation in comparison to control treatment. The lowest essence yield with value of 0.73 mL/m² was related to treatment of manure without recommended chemical fertilizers which had 33.42% increment as compared with control treatment. Alterations trend in order to increase consume amount of chemical fertilizer was ascending. Totally, among different treatments combinations in first cutting, the highest essence yield with rate of 3.043 mL/m² was related to usage of manure and 100% chemical fertilizers which had 84.03% augmentation in comparison to control treatment. Minimum yield

of essence with amount of 0.316 mL/m² was belonged to treatment of 100% chemical fertilizers without bio-fertilizers which had 34.98% reduction as compared with control treatment (Fig. 8).

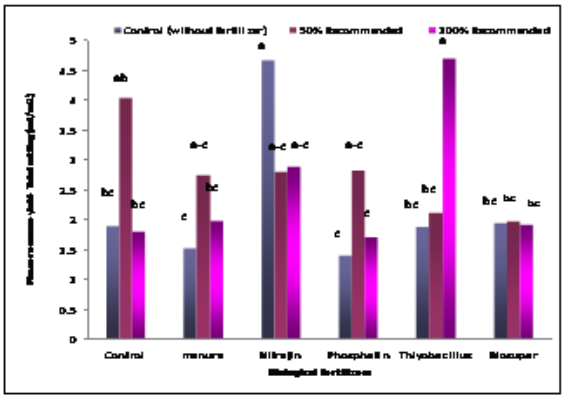

Fig. 10. The effect of biological fertilizers and amount of chemical fertilizers on flowers essence yield in third cutting.

Second cutting

Consume of chemical fertilizers in terms of non-use of bio-fertilizers had significant alterations toward each others. Among these treatments, the highest essence yield with rate of 3.22 mL/m² was related to treatment of 100% chemical fertilizers which had 33.85% augmentation in comparison to control treatment. The lowest essence yield with amount of 0.883 mL/m² was belonged to usage of 50% chemical fertilizers which had 58.54% decrement as compared with control treatment. In treatments with use of manure and different levels of chemical fertilizers; maximum yield of essence with amount of 2.307 mL/m² was achieved in use of manure with 50% recommended chemical fertilizers and had 7.67% augmentation in comparison to control treatment. The lowest essence yield with value of 1.24 mL/m² was related to treatment of manure with 100% recommended chemical fertilizers which had 41.78% reduction as compared with control treatment. Overall, among different treatments combinations in second moving, the highest essence yield with rate of 5.273 mL/m² was obtained in usage of Biosuper with 50% of recommended chemical fertilizers which had 59.61% augmentation in comparison to control treatment. Minimum yield of essence with amount of 0.54 mL/m² was belonged to treatment of Thiyobacillus without chemical fertilizers which had 74.65% decrement as compared with control treatment (Fig. 9).

Third cutting

Usage of chemical fertilizers in terms of non-use of bio-fertilizers had significant alterations toward each others. Among these treatments, the largest yield of essence with rate of 4.033 mL/m² was related to treatment of 50% chemical fertilizers which had 52.89% augmentation in comparison with control treatment. The least essence yield with amount of 1.807 mL/m² was belonged to consumption of 100% chemical fertilizers that had 4.9% decrement as compared with control treatment and of course had no significant difference with control treatment. In treatments with use of manure and different levels of chemical fertilizers; maximum yield of essence with amount of 2.75 mL/m², was achieved in consume of manure with 50% recommended chemical fertilizers and had 30.91% augmentation in comparison to control treatment. Minimum yield of essence with value of 1.523 mL/m² was related to treatment of manure without recommended chemical fertilizers which had 19.84% decrement as compared with control treatment. Totally, among different treatments combinations in third cutting, the highest essence yields were achieved in treatment of Thiyobacillus and 100% of recommended chemical fertilizers with rate of 4.703 mL/m² and also in treatment of Nitrajin without chemical fertilizers with value of 4.67 mL/m² which had 59.6% and 59.31% augmentations, respectively in comparison to control treatment. Minimum yield of essence with amount of 1.407 mL/m² was belonged to treatment of Phosphatin without chemical fertilizers which had 25.95% reduction as compared with control treatment (Fig. 10). Different levels of chemical fertilizers, nitrogen, phosphorus, potassium and biological fertilizers such as Azospirillum, phosphate solubilizing bacteria and mycorrhiza fungi on basil demonstrated that the highest essence yield and growth yield were achieved in combined treatment of 75% chemical fertilizer with biological fertilizer (Ajimoddin *et al.*, 2005).

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