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Original Article

The Effect of Bio Fertilizers, Manure and Chemical Fertilizer on Quantity and Quality of Essential Oil of Hyssop (*Hyssopus officinalis* L.)

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ABSTRACT

Hyssop is one of the medicinal plants that have many properties and benefits for the health of the body. Hyssop is an annual plant and often grows along roadsides. Hyssop leaves and green stems contain many bioactive compounds such as rosemary and Caffeic acid along with pinion, beta-pinna, limonene, pinoamophone and isopinocampone, tannins, glycosides, flavonoids, and essential oils. It is found that it can be produced purely by steam distillation from crushed leaves and stems. In this study, different bio fertilizers, chemical fertilizers, and manure were applied to find out their effects on the growth and active substances of Hyssop. Essential oils of Hyssop aerial parts were obtained by hydrodistillation and analysed by gas chromatography/mass spectrometry (GC/MS). Essential oil percent was maximum (1.66%) by application of N50P40K50 fertilizer and there was not any significant difference compared to control treatments. The highest value of oil yield (5.389 kg/ha) was achieved on cow manure treatment which increased oil yield compared to control (3.718 kg/ha). The highest value of isopinocampone (43.32%) was obtained from plants that received N50P40K50 fertilizer compare to the control treatment (39.32%). Control treatment had the highest value of pinocamphone (14.11%) compared with cattle manure treatment (11.59%).

INTRODUCTION

Medicinal plants are valuable resources in a wide range of natural resources that scientific identification, cultivation, development and proper utilization of them can be have very important role in community health, employment and non-petrol exports [1]. Quality of medicinal plants is more important than other crops. The effect of environmental factors is significant on quality and quantity of medicinal plants. Among the environmental effective factors, fertilizers and manure can be managed. These factors are affected on increase of yield and quality of yield of Cumin (*Cuminum cyminum*) [2].

Investigation showed that use of organic fertilizer had positive effect on growth and yield of *Mentha piperita* L., *Marrubium vulgar* L. and *Coriandrum sativum* L. [3-5]. Handavi, *et al*., (2010) was obtained that apply of manure fertilizer in soil was caused to increase the essence content and plant height of *Thymus* in the first cutting. But, in second cutting, all criteria were increased [6]. Also, manure was caused to provide of soil Nutrient elements, soil structure improves, moisture retention increases, creating suitable substrate for root growth, improve the quality and increase the yield of crops [1,7]. So, the effect of manure is significant on essential oil of Cumin (*C. cyminum*) and its constituents [2]. The use of 20 tons of manure per hectare had the best yield of flowering branches, and active ingredient in Hypericum [8]. The use of manure could significantly increase the plant height, number of branches, leaf yield, essential oil percent and essential oil yield of basil leaf (*Ocimum basilicum* L.) compared to control [9]. Use of manure in cumin (*C. cyminum*) caused a significant increase in biomass and plant height (Saeed Nejhad and Rezvani Moghadam, 2010). Cow manure increased sepal yield of Hibiscus tea plant (*Hibiscus sabdariffa* L.), and also their quality [10].

Nitrogen, phosphor and potassium (NPK) are the main elements needed for the plants. These elements were involved in all the biochemical processes in the energizing compounds, as well as in the energy transferring mechanisms. Additionally, NPK are a part of the cell protein, playing a particular role as a part of the cell protein, cell membrane, and nucleosides, which are responsible for reproduction and growth; despite its role in the herbal process and combination, the amount of phosphor in the herbal tissues is less than 0.1% of nitrogen. The damages resulting from the consumption of chemical fertilizers are reduction of the quantity and quality of crop – Boron aggregation, cadmium, and other heavy metals in the plant – reduction of absorption of copper, iron, and other microelements by the root – destruction of the soil structure [11].

Good soil fertility management ensures adequate nutrient availability to plants and increases yields. High above-ground biomass yield is obviously accompanied by an active root system, which releases an array of organic compounds into the rhizosphere [7]. It is well known that a considerable number of bacterial and fungal species possess a functional relationship and constitute a holistic system with plants. They can exert beneficial effects on plant growth [12]. Applying plant growth promoter bacteria such as nitrogen-fixing bacteria and phosphate solubilizing microorganisms in a sustainable agro-system has led to an increase in quality agricultural products especially medicinal plants [13]. Bio fertilizers are products containing living cells of different types of microorganisms [12,14] that have an ability to convert nutritionally important elements from unavailable to available form through biological processes [12] and are known to help with the expansion of the root system and better seed germination. Bio fertilizers differ from chemical and organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion, including the plant growth promoting rhizobacteria (PGPR) such as *Azospirillum*, *Azotobacter*, *Pseudomonas fluorescens*, and several gram positive *Bacillus* spp. [14]. The diastrophic rhizobiocoenosis is an important biological process that plays a major role in satisfying the nutritional requirements of commercial medicinal plants [15] the strong and rapidly stimulating effect of fungal elicitor on plant secondary metabolism in medicinal plants has attracted considerable attention and research efforts [16]. *Azotobacter* and *Azospirillum* are free-living N_2-fixing bacteria that in the rhizospheric zone have the ability to synthesize and secret some biologically active substances that enhance root growth. They also increase germination and vigor in young plants, leading to improved crop stands [14] Phosphate solubilizing microorganisms such as; bacteria and fungi are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization [14]. Also, by using bio fertilizers, the quantity and quality of active substances of medicinal plants can be improved [17, 18]. Some studies have reported that plant growth promoter bacteria such as Azotobacter chroococcum, Azospirillum lipoferum, Bacillus sp and Pseudomonas sp could cause increased growth and yield of medicinal plants such as rosemary [19], fennel [18,20,21], turmeric [22], mint [23], hyssop [1], geranium [24], marjoram [25-27] davana [28], dragonhead [29], black cumin [30], dill [31], thyme [32] and basil [33,34].

Hyssop (*Hyssopus officinalis*) is an herbaceous plant of the genus Hyssopus native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea. H. officinalis L. is an evergreen Shrub the flowers are hermaphrodite (have both male and female organs) and are pollinated by Bees. Hyssop is a brightly colored shrub or subshrub that ranges from 30 to 60 cm in height. The stem is woody at the base; several straight branches are grown from it. Its leaves are lanceolate, dark green in color, and from 2 to 2.5 cm long. During the summer, the plant produces bunches of pink, blue, or, more rarely, white fragrant flowers. Herb hyssop leaves are used as an aromatic condiment. The leaves have a slightly bitter taste due to their tannins, and an intense minty aroma. It is used moderately in cooking due to its intensity. The herb is also used to flavor liqueur and is part of the official formulation of Chartreuse. The plant also includes the chemicals thujone and phenol, which give it antiseptic properties [35]. A strongly aromatic flavor, somewhat like a cross between sage and mint, it has fallen out of favor in recent years. It has a positive effect when used to treat bronchitis and respiratory infections, especially where there is excessive mucous production [36]. Hyssop can irritate the mucous membranes, so it is the best given after an infection has peaked when the herb's tonic action encourages a general recovery [37]. The leaves and flowering tops are antiseptic, antitussive, astringent, carminative, diaphoretic, emmenagogue, expectorant, pectoral, sedative, stimulant, stomachic, tonic and vasodilator. It is commonly used as an aromatic herb and medicinal plant [38]. The plant can be harvested when in full flower and dried for later use. A tea made from the leaves is used in the treatment of flatulence, stomachaches, upper respiratory tract infections, coughs in children, etc. A poultice made from the fresh herb is used to heal wounds. The essential oil is used in aromatherapy. This oil should not be used on people who are highly strung as it can cause epileptic symptoms. The essential oil should not be used internally except under professional supervision.

This research aimed to study the effects of bio-fertilizers, chemical fertilizer and cow manure on the growth and essential oils of Hyssop.

MATERIAL AND METHODS

This study was carried out during 2009-2011 on a land with an area of 2000m in Arak on a field with geographical coordinates: latitude: 34º- 5', longitude: 49º- 42', and height of 1787 m above the sea level.

Treatments containing N-fixing containing Azotobacter; and Azospirillum with 10^8 CFU viable cell per ml (Nitrogen), Phosphate solubilizing containing bacteria Bacillus coagulans; and Glomus fungus with 2× 10^6 active Propagal of fungus (Barvar2), Chemical fertilizers $N_{50}P_{40}K_{50}$ about 1/2 recommended (urea 46%, triple superphosphate 48% and potassium sulphate 52%), cattle manure (40 ton/ha) and control. Before plant culture, the soil was analyzed for determination of texture and macro element components (Table 1).

The suitable land was chosen and after preparation of the land, the seeds were planted in the plot. The depth of seeds planted on the furrow was 1 to 2cm. The intervals between plants are considered 40cm. Generally, the number of plants on each plot is considered 50. Based on the soil test the amount of nitrogen and potassium fertilizers needed were 50 kg per hectare and phosphor fertilizer 40 kg per hectare. Of course, the seeds before being planted were saturated with Components of Phosphate solubilizing bacteria (Barvar2) and Nitrogen fixing bacteria (Nitrogen) as per the prescribed method. Sampling for measuring the physiological procedure was commenced 25 days upon greening and was repeated once every week.

Sampling was carried out in such a way: two sidelines were removed from each plot due to marginal impacts, and after removing one bush from the three middle rows (from both ends of cultivated rows), 6 bushes were picked from each plot for phonology and 10 bushes were sent to the lab to measure the percentage of the essence (quantitative). After collecting samples, their roots, stems, leaves and twigs were separated and each weighed individually. Then for 72 hours were placed in the oven at 75 °C. After being dried, the samples were weighed again. After harvesting, several samples were dried in shadow and extraction was performed in the laboratory. To measure the essential oil amount, the Clevenger apparatus and water distillation method were used; 100 grams of leaf powder and the dried shoot were poured into a 1-liter flask of the device and 800 ml water was added to it and heated for 2 hours. Savory essential oil is a colorless or yellowish liquid and its density is 0.895 – 0.913. Extraction of essential oil did with three replications.

Table 1 Result of field soil Test

Depth	% of saturation	Ec (ds/m)	pH	T.N.V	O.M (%)	P (%)	K (%)	N (%)	Sand (%)	Silt (%)	Clay (%)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)
0-30	37	0.6	8	28	0.61	11.4	434	6.2	52	36	12	10.49	2.16	2.96	7.88

Table 2 Analysis of manure sample (cow rotten manure) used in the study

C/N ratio	Mn (ppm)	Pb (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)	O. M (%)	N (%)	Ca (%)	K (%)	P (%)	pH	Ec (ds/m)
16.8	.298	.016	0.05	0.17	4.39	31.4	1.87	1.53	2.38	0.69	7.64	20.3

Gas Chromatography analysis was performed on an Agilent technologist model (6990 USA) series II gas chromatograph equipped with a flame ionization detector and capillary column HP-5 (30 m×0.25 mm, 0.25 µm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 240 ºC at a rate of 3 ºC/min. The injector and detector temperatures were 240 and 250 ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using the split sampling technique by a ratio of 1:20. Quantitative data obtained from electronic integration of essential oil was also analyzed by Hewlett- Packard GC-MS (model 6890 series II) operating at 70e V ionization energy. The device was equipped with an HP-5 capillary column (phenyl methyl siloxane (30 m × 0.25 mm, 0.25 µm film thickness) with Him as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra. Statistical analysis of the data was carried out based on randomized complete block and comparing their average by (LSD test at $p < 0.05$), analyzing the data using SAS 9.1 software.2018

RESULTS AND DISCUSSION

Plant Height

The results have indicated that plant height was significantly affected by the application of different treatments of fertilizers at 1% level (Table 3).

The highest plant height (48.7 cm) was obtained by applying cow manure and the lowest plant height (38.8 cm) was indicated by control treatment and between the two treatments was the significant differences at 0.05 levels (Table4). According to the analysis, NPK fertilizers and manure have increased plant height by enhancing the nitrogen content and the rate of photosynthesis [39]. In general, the best treatment was the high application of animal manure for best yield and for other medicinal plants in local conditions [1]. The application of manure in onion increased plant height by 17.41% compared to the control [21]. The use of manure could significantly increase the plant height of basil leaf (*O. basilicum* L.) compared to the control [9]. The use of manure in cumin (*C. cyminum*) caused a significant increase in plant height. [40].

Root Length

The results have indicated that root length wasn't significantly affected by application of different treatments of fertilizers (Table 3).

The numbers are shown with the same letters are statistically no significant difference at 5% level

The highest root length (11.89 cm) was obtained by applying cow manure and the lowest root length (9.89 cm) was indicated by control treatment and between the two treatments were not significant difference in the level of 0.05 (Table4).

Number of Stems

The results have indicated that number of stems was significantly affected by the application of different treatments of fertilizers at 5% level (Table 3). The highest number of stems (14.78) was obtained by applying chemical fertilizer ($N_{50}P_{40}K_{50}$) and the lowest number of stems (11.22) was indicated by control treatment and between the two treatments were significant difference in the level of 0.05 (Table4). According to the present analysis, NPK fertilizers, Nitrogen and Manure have increased number of stems. The use of manure could significantly increase the number of branches of basil leaf (*O. basilicum* L.) compared to control [9].

Flower shoot fresh weight

The results presented in Table 3 have revealed that studied various treatments didn't have significant effects on flower shoot fresh weight. The maximum flower shoot fresh weight (15.09 g) was obtained by using integrated application of chemical fertilizer ($N_{50}P_{40}K_{50}$) and the minimum flower shoot fresh weight (12.17 g) was indicated in control and between the two treatments were significant difference in the level of 0.05. According to the present analysis, NPK fertilizers and Manure have increased flower shoot fresh weight (Table 4).

Flower Shoot Dry Yield

The Study of analyze variance table showed that treatments didn't have a significant effect on flower shoot dry yield (Table 3). Among the treatments Manure with an average of 335.4 g had highest and control with 245.9 g had least amount and between the two treatments were significant difference in the level of 0.05. Results show that application manure had leaded in to a significant increase in flower shoot dry yield compare to control (Table4). The use of 20 tons of manure per hectare had the best yield of flowering branches, and active ingredient in Hypericum [8]. The use of manure could significantly increase leaf yield, of basil leaf (*Ocimum basilicum* L.) compared to control [9]. Cow manure increased sepal yield of Hibiscus tea plant (*Hibiscus sabdariffa* L.), and also their quality [10].

Dry biological yield

The results presented in Table 3 have demonstrated that dry biological yield was influenced by the application of different treatments of fertilizers, significantly at 5% level. Among various treatments, treatment of the application of cow manure (2827 kg/ha) have indicated maximum increase in dry biological yield and treatment of control (2088 kg/ha) have indicated minimum in dry biological yield and between the two treatments were significant difference in the level of 0.05. Results show that application manure had leaded in to a significant increase in dry biological yield compare to control (Table 4). Organic manures in combination with inorganic fertilizers render greater beneficial effects on plant growth and yield [41] (Channabasanagowda *et al*., 2008). The use of manure could significantly increase the leaf yield of basil leaf (*O. basilicum* L.) compared to control [9].

Table 3 Analysis of the effect of bio fertilizers, fertilizer and manure on characteristics of hyssop

Source of variation (SOV)	Year (Y)	Error a (EY)	Treatments (T)	Y×T	Error b (ET)	Cv (%)
Degrees of freedom (df)	2	6	4	8	24	----
Plant height	563.95 **	13.722	144.579 **	33.596 *	13.7	8.49
Root length	7.0222 ns	1.3111	6.58888 ns	1.3556 ns	4.1	8.18
Number of branches	69.756 **	10.133	19.0333 *	4.0333 ns	4.8	6.79
Flower shoot wet weight	16.991 ns	12.659	17.7008 ns	3.3622 ns	7.1	9.71
Flower shoot dry yield	7900.8 ns	9302.9	12317.8 ns	1495.4 ns	5264.9	14.96
Essential oil	0.0578 ns	0.5195	0.02848 ns	0.0810 ns	0.1	10.72
Biological yield	3222937 *	533731	871112.7 *	83481.9 ns	217610	9.55
Essential oil yield	3.2086 ns	5.0349	4.83785 *	1.4406 ns	1.3	15.38

Ns and *, ** non-significant, significant at 5% and 1% respectively.

Table 4 mean comparison of hyssop properties under effect of bio-fertilizers, fertilizer and manure

Properties	Year1	Year2	Year3	LSD	Control	Barvar2	Nitrogen	$N_{50}P_{40\ 50}$	Manure	LSD
Plant height (cm)	43.1 b	37.6 c	49.9 a	2.8	38.8 b	41.6 b	42 b	46.6 a	48.7 a	4.2
Root length (cm)	11.7 a	10.4 a	11.3 a	1.5	9.9 a	10.6 a	11.5 a	11.8 a	11.9 a	1
Number of stems	12.3 b	11.3 b	15.47 a	1.65	11.22 c	11.89 bc	13.5 ab	14.7 a	13.78 ab	5.676
Flower S.W.W (g)	14.0 a	12.3 a	14.33 a	2.01	12.17 b	12.20 b	13.5ab	15.09a	14.8 ab	0.521
Flower S.D.Y (g)	292 a	267 a	312.9 a	54.6	245.9 b	262.6 ab	292.4ab	316.8ab	335.4 a	0.984
Essential oil (%)	1.5 a	1.63a	1.593 a	0.24	1.50 a	1.554 a	1.58 a	1.66a	1.594 a	0.127
Biomass (kg/ha)	2855 a	1928 c	2373 b	351	2088 b	2098 b	2530 ab	2383ab	2827 a	194.7
Essence yield(kg/h)	4.2 a	4.38 a	5.070 a	0.86	3.718 b	3.912 b	4.57 ab	5.137a	5.389 a	3.860

Table 5 The chemical components of hyssop oil with different treatments of fertilizers

Row	RI	Compound name	Control	Barvar2	Nitrogen	$N_{50}P_{40}K_{50}$	Manure
1	928	α-thujene	0.455	0.851	0.653	0.548	0.627
2	935	α-pinene	1.205	0.315	0.859	1.871	1.544
3	978	Sabinene	0.625	0.734	0.523	0.854	0.629
4	987	β-pinene	1.711	0.834	1.235	1.678	1.539
5	992	Myrcene	0.305	0.512	0.439	0.523	0.546
6	997	n-decane	8.615	7.629	7.918	6.452	6.613
7	1012	δ-3-carene	1.104	1.518	1.206	1.135	1.218
8	1029	P-cymene	0.565	2.81	2.124	2.019	1.993
9	1035	Limonene	0.485	0.422	0.417	0.453	0.448
10	1045	(Z)-β-ocimene	1.151	0.954	0.978	0.937	1.124
11	1054	(E)-β-ocimene	0.615	0.412	0.433	0.461	0.491
12	1107	Linalool	0.633	0.717	0.463	0.628	0.792
13	1136	Camphor	0.535	0.343	0.656	0.549	0.445
14	1158	Pinocomphone	14.112	12.599	11.961	11.875	11.587
15	1165	Pinocarvone	6.053	6.491	5.107	5.128	5.542
16	1178	Iso pinocamphone	39.316	40.227	42.127	43.315	42.958
17	1192	Terpinen-4-ol	1.835	1.723	1.654	1.435	1.265
18	1203	α-terpineol	0.295	0.543	0.687	0.556	0.458
19	1212	Myrtenol	1.713	2.012	2.081	1.618	1.522
20	1236	Carvon	0.355	0.289	0.234	0.235	0.312
21	1245	Cumin aldehyde	0.565	0.314	0.345	0.564	0.485
22	1236	Carvon	0.355	0.314	0.423	0.348	0.389
23	1263	Piperitone	1.495	1.77	0.876	0.568	0.879
24	1289	Thymol	0.667	0.634	0.866	0.565	0.425
25	1306	Carvacrol	2.755	3.022	2.729	2.156	2.423
26	1348	Eugenol	0.715	0.534	0.419	0.373	0.395
27	1375	β-bourbonene	1.455	1.628	1.312	1.668	1.921
28	1396	Methyl eugenol	0.335	0.305	0.423	0.542	0.445
29	1417	α-gurjunene	0.365	0.417	1.113	1.322	1.415
30	1425	β-caryophyllene	0.365	0.359	0.411	0.348	0.328
31	1466	α-humulene	0.092	0.112	0.121	0.151	0.214
32	1467	Allo aromadendrene	0.455	0.345	0.489	0.516	0.571
33	1482	Germacrene D	0.625	0.563	0.689	0.712	0.735
34	1537	Elemol	0.655	0.585	0.593	0.548	0.483
35	1568	Spathulenol	0.089	0.292	0.283	0.212	0.318
36	1582	Caryophyllene oxide	0.575	0.621	0.635	0.568	0.598
37	1675	α-bisabolol	1.555	1.567	1.544	1.524	1.658
-	-	Total (%)	94.801	95.317	95.026	94.955	95.335

Use of manure in cumin (*C. cyminum*) caused a significant increase in biomass [40]. Cow manure increased sepal yield of Hibiscus tea plant (*Hibiscus sabdariffa* L.), and also their quality [10].

Essential Oil Percent

The Study of analyze variance table showed that treatments didn't have a significant effect on essential oil percent (Table 3). Among the treatments, $N_{50}P_{40}K_{50}$ fertilizer with an average of 1.661% had highest and control with 1.501% had least amount and between the two treatments weren't significant difference in the level of 0.05 (Table 4).

Essential Oil Yield

The Study of analyze variance table showed that treatments had a significant effect on Essential oil yield at 5% level (Table 3). Between the treatments cow manure with a mean of 5.389 kg/ha had highest and control with 3.718 kg/ha had least amount and between the two treatments were significant difference in the level of 0.05 (Table 4).

Results show that application cow manure, $N_{50}P_{40}K_{50}$ fertilizer and Nitrogen had leaded in to a significant increase in essential oil yield compare to control. The use of manure could significantly increase essential oil yield of basil leaf (*O. basilicum* L.) compared to control [9]. Some studies have reported that plant growth promoter bacteria such as Azotobacter chroococcum, Azospirillum lipoferum, could cause increased growth and yield of medicinal plants such as rosemary [19], fennel [21], turmeric [22], mint [23], hyssop [1], geranium [41], marjoram [27], davana [28], dragonhead [29], black cumin [30], dill [31], thyme [32] and basil [34].

The Chemical Composition of Essential Oils

Results obtained from qualitative and quantitative analysis of essential oils shown in Table Thirty - seven compounds representing over 94.0% of the essential oils identified (Table 5).

To study the effect of Barvar2, Nitrogen, Manure and $N_{50}P_{40}K_{50}$ fertilization on the main constituents of the essential oil, the oil of each treatment was separately subjected to gas liquid chromatography and the main compounds and their relative percentages are shown in (Table 5). Iso pinocamphone, a phenolic compound, found to be the first major compound and ranged from 39.32 to 43.32%. Its maximum content observed in the essential oil of the herb that received $N_{50}P_{40}K_{50}$ fertilizer (43.32%) and its minimum content observed in the essential oil of the herb that received control (39.32%). Iso pinocamphone content of Hyssop herb increased with the use of manure, $N_{50}P_{40}K_{50}$fertilizer and bio fertilizers treatments compared to the control.

The second main compound identified Pinocomphone. Which ranged from its maximum content (14.11%) received control treatment to its minimum relative percent (11.59%) with cattle manure treatment. The use of $N_{50}P_{40}K_{50}$ fertilizer, Manure and bio fertilizers treatments decrease Pinocomphone content of Hyssop herb compared to the control.

The third main compound identified N-decane. Which ranged from its maximum content (8.62%) received control treatment to its minimum relative percent (6.45%) with $N_{50}P_{40}K_{50}$ fertilizer treatment. The use of $N_{50}P_{40}K_{50}$ fertilizer, Manure and bio fertilizers treatments decrease N-decane content of Hyssop herb compared to the control.

The quality and quantity of the materials forming H. *officinalis* L. essential oil had some differences and similarities with the cases reported in other regions. The essential oil of H. officinalis L. plant has been widely studied in Iran and other countries. In the literature, isopinocamphone, pinocamphone N-decane and pinocarvone were reported to be the most abundant components in hyssop oil [42]. The composition of the essential oil of H. *officinalis* has been examined previously by [43], [44], [45], [46], and [47].

CONCLUSION

Hyssop (*H. officinalis*) is an herbaceous plant and an aromatic and medicinal plant belonging to the family Lamiaceae used in the Iranian folk medicine for various purposes. Our results showed that plant height, number of stems, biomass and essential oil yield in hyssop significantly increased by application of manure, bio fertilizer (Nitrogen) and chemical fertilizer ($N_{50}P_{40}K_{50}$) treatments compared to control. Thirty-seven components identified in H. *officinalis* essential oils. Some component such as iso pinocamphone, pinocamphone N-decane changed with using manure, bio fertilizers (Barvar2 & Nitrogen) and fertilizer ($N_{50}P_{40}K_{50}$). The results of this study can be useful to investigate the use of manure, bio fertilizers and fertilizer on yield and bioactive component in medicinal plants.

REFERENCES

1. Koocheki A., Sabet Teimouri M. Effects of fertilizer types and irrigation intervals of on quantity criteria of Lavender (*Lavandula angustifolia*), Rosemary (*Rosemarinus Officinalis*) and Hyssop (*Hyssopus Officinalis*). Iranian Journal of Field Crops Research. 2011; 9(15); 78-87.
2. Ahmadian A., Tavassoli A., Amiri E. The interaction effect of water stress and manure on yield components, essential oil and chemical compositions of Cumin (*Cuminum cyminum*). African Journal of Agricultural Research. 2011; 6(10): 2309-2315.
3. Chatterjee S.K. Cultivation of Medicinal and Aromatic Plants in India- a Commercial Approach. Proceeding of an International Conference on MAP. Acta Horticulture (ISHS). 2003; 576:191-202.

4. Delate K. Heenah Mahyah Student Farm Herb Trial. Leopold Center for Sustainable Agriculture Annual Reports, Iowa State University, Ames, IA. 2000.
5. Griffe P., Metha S., Shankar D. Organic Production of Medicinal, Aromatic and Dye-Yielding Plants (MADPs): Forward, Preface and Introduction. FAO. 2003.
6. Mandal A., Patra A.K., Singh D., Swarup A., Ebhin Masto R. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bio-Resource Technology. 2007; 98: 3585-3592.
7. Migahed H.A., Ahmed A.E., Abdel Ghany B.F. Effect of different bacterial strains as bio fertilizer agents on growth, production and oil of Apium grave lens under calcareous soil. Arab Universities J. Agric. Sci. 2004; 12(2): 511-525.
8. Tanami Zarandi S.M.K., Rezvani Moghadam P, Jahan M. Comparison the effect of organic and chemical fertilizers on yield and essential oil percentage of basil (*Ocimum basilicum* L.). Journal of Agroecology. 2010; 2:70-82. (In Persian)
9. Gendy A.SH., Said-Al Ahl H.A.H., Abeer A.M. Growth, Productivity and Chemical Constituents of Roselle (*Hibiscus sabdariffa* L.) Plants as Influenced by Cattle Manure and Biofertilizers Treatments Australian Journal of Basic and Applied Sciences. 2012; 6(5):1-12.
10. Allahverdi A.R., Alidoost Gh., Akbari A., Ghasemi A. study on the impact of various quantities of composite fertilizer and recycling of urban waste, N and P on growth, performance and absorption of P in corn. Excerpts of the 3rd National Conference on Application of Organic Substances and Optimum Use of Fertilizers and Poisons in Agriculture, Agriculture Research and Training Organization, 2003;276-283
11. Vessey J.K. Plant growth promoting rhizobacteria as bio fertilizers. Plant Soil. 2003; 255: 571-586.
12. Sharma A.K. Biofertilizers for sustainable agriculture. Agrobios, India.2002; 407
13. Chen Y.P., Rekha P.D., Arun A.B., Shen F.T. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil. Ecol.2006; 34: 33-41.
14. Deka B.C., Bora G.C., Shadeque A. Effect of Azospirillum on growth and yield of chilli (*Capsicum annuum* L.) cultivar Pusa Jawala, Haryana. J. Hort. Sci. 1992; 38:41–46.
15. Delate K. Heenah Mahyah Student Farm Herb Trial. Leopold Center for Sustainable Agriculture Annual Reports, Iowa State University, Ames, IA. 2000.
16. Rashmi K.R., Earanna N., Vasundhara M. Influence of bio fertilizers on growth, biomass and biochemical constituents of *Ocimum gratissimum*. L. Biomed. 2008; 3(2): 123-130.
17. Saeed Nejhad A.H., Rezvani Moghadam P. Evaluation of consumption of compost, vermicompost and manure fertilizers on yield, yield components of Cumin and essence percentage. Horticulture sciences journal. 2010;24(2): 142-148.
18. Abdel-Aziz M., Pokluda R., Abdel-Wahab M. Influence of compost, microorganisms and NPK fertilizer upon growth, chemical composition and essential oil production of *Rosmarinus officinalis* L. Not. Bot. Hort. Agrobot. Cluj. 2007; 35(1): 86-90.
19. Abdel-Hadi Nadia I.M., Aboel-Ala H.K., Abdel-Azim W.M. Response of Some Mentha Species to Plant Growth Promoting Bacteria (PGPB) Isolated from Soil Rhizosphere. Australian Journal of Basic and Applied Sciences. 2009; 3(4): 4437-4448.
20. Moradi R., Nasiri Mahallati M., Rezvani Moghaddam P., Lakzian A., Nejad Ali A. The effect of application of organic and biological fertilizers on quantity and quality of essential oil in fennel (*Foeniculum vulgare*). J. Hortic. Sci. 2011; 25(1): 25-33.
21. Velmurugan M., Chezhiyan N., Jawaharlal M. Influence of organic manures and inorganic fertilizers on cured rhizome yield and quality of turmeric (*Curcuma longa* L.) cv. BSR-2. International J. Agric. Sci. 2008; 4(1): 142-145.
22. Abdel-Hadi Nadia I.M., Aboel-Ala H.K., Abdel-Azim W.M. Response of Some Mentha Species To Plant Growth Promoting Bacteria (PGPB) Isolated From Soil Rhizosphere. Australian Journal of Basic and Applied Sciences. 2009; 3(4): 4437-4448.
23. Leithy S., Gaballah M.S., Gomaa A.M. Associative impact of bio- and organic fertilizers on geranium plants grown under saline conditions. International Journal of Academic Research. 2009; 1(1): 17-23.
24. Lebaschi M.H, Matin A, Sharifi-Ashurabadi A. Comparing the agricultural and natural ecosystems in the production of hypericin. Research and Construction in Natural Resources. 2004;16(2):48-54.
25. Al-Fraihat A.H., Al-dalain S.Y.A., Al-Rawashdeh Z.B., Abu-Darwish M.S., Al-Tabbal J.A. Effect of organic and bio fertilizers on growth, herb yield and volatile oil of marjoram plant grown in Ajloun region, Jordan. Journal of Medicinal Plants Research. 2011; 5(13): 2822-2833.
26. Kumar T.S., Swaminathan V., Kumar S. Influence of nitrogen, phosphorus and bio fertilizers on growth, yield and essential oil constituents in ratio on crop of davana (*Artemisia pallens* Wall.). Electronic J. Environ. Agric. Food Chemistry. 2009; 8(2): 86-95.
27. Rahimzadeh S., Sohrabi Y., Heidari G.R., Eivazi A.R., Hoseini T. Effect of bio and chemical fertilizers on yield and quality of dragonhead (*Dracocephalum moldavica* L.). Iranian Journal of Medicinal and Aromatic Plants. 2011; 27(1): 81-96.
28. Valadabadi S.A., Farahani H.A. Investigation of bio fertilizers influence on quantity and quality characteristics in Nigella sativa L. J. Hortic. Forestry. 2011; 3(3): 88-92.
29. Darzi M.T., Haj Seyed Hadi M.R., Rejali F. Effects of the application of vermicompost and nitrogen fixing bacteria

on quantity and quality of the essential oil in dill (*Anethum graveolens*). J. Medicinal Plants Res. 2012; 6(21): 3793-3799.

30. Yadegari M., Farahani G.H.N., Mosadeghzad Z. Biofertilizers effects on quantitative and qualitative yield of Thyme (*Thymus vulgaris*). African J Agric Res. 2012; 7(34): 4716-4723.
31. Veres, K., Varga, E., Dobos, A., Hajdn, Z., Mathe, I., Pluhar,Z.Nemeth, E. and J. Bernath (1997). Investigation of thecomposition of essential oils of *Hyssopus officinalis* L.populations, 217-220 pp. In: Ch. Franz, A. Mathe, G. Buchbaner (Eds), Essential Oils: Basic and Applied Research, Allured Publishing Corporation, Carol Stream, IL, pp. 217220.
32. Jahan M., Amiri M.B., Dehghanipoor F., Tahami M.K. Effects of Biofertilizers and Winter Cover Crops on essential oil production and some agroecological characteristics of basil (*Ocimum basilicum* L.) in a organic agrosystem. Journal of Iranian Agronomy Researches. 2013; 10(4): 751-763.
33. Van Wyk V., Michael W. Medicinal Plants of the World.2004; P. 177.
34. Chevallier A. Encyclopedia of Medicinal Plants. Dorling Kindersley Publishing Australia. 2001;336 p.
35. Chiej R. The Macdonald Encyclopedia of Medicinal Plants. London, Macdonald & Co. 1984; P. 274.
36. Mills S., Bone K. The Essential Guide to Herbal Safety. Philadelphia, Elsevier Churchill Livingstone. P. 376. 2005.
37. Kumar T.S., Swaminathan V., Kumar S. Influence of nitrogen, phosphorus and bio fertilizers on growth, yield and essential oil constituents in ratio on crop of davana (*Artemisia pallens* Wall.). Electronic J. Environ. Agric. Food Chemistry. 2009; 8(2): 86-95.
38. Saeed Nejhad A.H., Rezvani Moghadam P. Evaluation of consumption of compost, vermicompost and manure fertilizers on yield, yield components of Cumin and essence percentage. Hortic Sci J. 2010; 24(2) 142-148.
39. Hendawy S.F., Azza A., Ezz E.D., Eman EA, Omer EA. Productivity and Oil Quality of Thymus Vulgaris L. Under Organic Fertilization Conditions Ozean Journal of Applied Sciences. 2010 ;3(2):203-216.
40. Svoboda K.P.B., Galambosi S.G., Deans Hethelyi E. Agronomical and biochemical investigation of *Hyssopus officinalis* L. from various geographical sources. Acta Hort. 1993; 344:434-443.
41. Veres K., Varga E., Dobos A., Hajdn Z., Mathe I., Pluhar Z., Nemeth E., Bernath J. Investigation of thecomposition of essential oils of Hyssopus officinalis L.populations, 217-220 pp. In: Ch. Franz, A. Mathe, G. Buchbaner (Eds), Essential Oils: Basic and Applied Research, Allured Publishing Corporation, Carol Stream, IL1997, pp. 217220.
42. Vallejo M., Herraiz J., Perez-Alonso M., Velasco Negueruela A. Volatile oil of *Hyssopus officinalis* L.from Spain. J. Essent. Oil Res. 1995; 7:567-568.
43. De Martino L., De Feo, V., Nazzaro F. Chemical composition and in vitroantimicrobial and mutagenic activities of seven Lamiaceae essential oils. Molecules. 200914, 4213–4230.
44. Ozer H., Sokmen M., Gulluce M., Adiguzel A., Kilic H., Sahin F., Sokmen A., Baris O. In VitroAntimicrobial and antioxidant activities of the essential oils and methanol extracts of *Hyssopus officinalis* L. SSP. Angustifolius. Ital. J. Food Sci.2 (18): 73-83.
45. Kizil S, Toncer O., Ipek A., Arslan N., Saglam S., Khawar K.M. Blooming stages of Turkish hyssop (*Hyssopus officinalis* L.) affect essential oil composition Acta Agriculturae Scandinavica, Section B-Soil & Plant Science. 2008; 58(3): 273-279.