Journal of Herbal Drug

journal homepage: www.jhd.iaushk.ac.ir

Effects of different sources of nitrogen fertilizer and applied rates on essential oil content and composition of peppermints

Adel Poshtdar[1*], Alireza Abdali Mashhadi[1], Foad Moradi[2], Seyed Ata Siadat[1], Abdolmehdi Bakhshandeh[1]

[1]*Department of Agronomy, Ramin Agriculture and Natural Resources University of Khouzestan, Khouzestan, Iran;*
Email: *adelposhtdar@gmail.com
[2]*Agricultural Biotechnology Research Institute of Iran, Karaj, Iran;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *February 24th 2015*
Accepted *May 26th 2015*

Key words:

- *GC/MS*
- *Menthapiperita*
- *Menthol*
- *Phytochemical*
- *UAN*

ABSTRACT

Background & Aim: Peppermint known for its healing properties is a plant from the Labiatae family. Currently, different rates of nitrogenous fertilizers are used in production of peppermint while there is no precise information available about how much of nitrogen fertilizer is the optimum rate for this plant. The main purpose of this research was to evaluate the response of peppermint chemical composition to different nitrogen rates and type of nutrition strategy (with control and conventional chemical sources).

Experimental: Samples collected from different mint nutrition strategy [conventional: four nitrogen levels from three fertilizers source (UAN, urea and ammonium sulfate), and control (no added fertilizer) in the Khouzestan province at the southwest of Iran.

Results: Nitrogen supply led to increase of fresh and dry yield of peppermint. Maximum essential oil obtained from 210 kgN/ha of nitrogen fertilizer in UAN which showed the best results compared to urea, ammonium sulfate and control. The GC/MS data showed that the qualitative of the components appeared to be not constant in different nutrient strategy. Moreover, remarkable variations were found in the amounts of the major of essential oil constituents. A total thirty-five components, comprising 98.87, 97.62, 98.09 and 97.98% of the obtained total essential oils were characterized in control, UAN, urea and ammonium sulfate samples, respectively. Also Menthol (41.22, 33.7, 30.62 and 33.23%), menthone (16.32, 26.25, 27.33 and 26.7 %), menthofuran (4.09, 4.74, 5.81 and 5.14 %) and isomenthone (6.47, 7.48, 7.26 and 7.43%) were the main components of peppermint oil.

Recommended applications/industrie: Since, essential oil of peppermint, particularly menthol component, has many application in pharmaceutical and perfumery industry due to antiviral, antibacterial and antispasmodic activity, UAN fertilizers is recommended to be used in addition to control in view of the increasing in quality and quantity of essential oil.

1. Introduction

Consumer demand for organic foods has continuously increased because of the perception that they might contain greater amounts of beneficial components than their conventionally produced counterparts. Conflicting findings on the quality and nutritional values of organic foods compared to their conventional counterparts have been reported. For instance, a human crossover intervention study involving 16 subjects demonstrated that organic and conventional fruits and vegetables differed in their concentrations of five selected flavonoids and resulted in different urinary excretions of the major dietary flavonoids (Grinder-Pedersen *et al*., 2003). In contrast, a systematic review concluded that there was no evidence indicating the nutrition quality difference between organic and conventional foods (Dangour *et al*., 2009). Mints comprise a group of species of the genus *Mentha* belonging to the family Lamiaceae. The aerial parts of the herb on distillation yields essential oil containing a large number of aroma chemicals like menthol, menthone, isomenthone, menthofuran, carvone, linalool, linalyl acetate and piperitenone oxide which are used in pharmaceutical, food, flavour, cosmetics, beverages and allied industries (Verma *et al*., 2010). Peppermint (*Mentha x piperita* L.) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone. Peppermint oil is used for flavouring pharmaceuticals and oral preparations. The annual world production of *Menthaarvensis* L. and *M. piperita* L. oils are 22,000 and 7500 mt, while India is producing 16000 and 100 mt per year, respectively (Khanuja, 2007; Patra *et al*., 2002). And it was found that the chemical composition is influenced by various factors, such as geographical location (Murray *et al*., 1988), environmental conditions (Chalchat *et al*., 1997) and agro-climatic requirements of the crops (Chand *et al*., 2004).Cultivation techniques have a great influence on plant growth (biomass production) and the quality and quantity of secondary metabolites. There is an absolute N requirement for plant growth, and crop yields and quality depend upon substantial N inputs. Chemical N fertilizers were first used in agriculture in the 19th century, and subsequently to a much greater extent after the development of the Haber Bosch process at the beginning of the 20th century. At the present time, more than half of the chemically fixed N is used by agriculture, amounting to in excess of 80 Mt per year, worldwide (Hawkesford, 2014). Most authors report that significantly higher dry matter and essential oil yields for corn mint, peppermint and spearmint were obtained with higher nitrogen application but that the application of nitrogen did not affect the chemical composition of the essential oil (Saxena & Singh, 1995). Other authors have reported that a decrease in menthol and an increase in menthone and menthyl acetate in peppermint and corn mint has been found with increased application of N (Duhan *et al*., 1977; Hornok, 1983). According to Kothari & Singh (1995), carvone and limonene concentrations in Scotch spearmint (*M. gracilis*) were affected by levels of N, and in general, an increase of N decreased carvone but increased limonene in oil. This is not quite in agreement with Singh & Singh (1986) who reported that the application of N increased carvone in spearmint oil. Marotti and co-authors (1994) used different levels of nitrogen and phosphorus in peppermint trials. They reported that mineral fertilization seemed to increase the menthol content of essential oil compared with untreated plants. Hornok (1983) reported that the most effective treatment on yield of peppermint regarding to fertilizers was achieved first by the level of nitrogen and second by the level of calcium. Phosphorus fertilization had no effect on yield. However, a high quality of peppermint oil in any region requires the optimum use of fertilizers and water to maintain herbage growth and delay maturity as long as possible so that the herbage may be harvested with a minimum of flowers (Murray *et al*., 1988).

Nitrogen may be applied in various forms to fertilization strategies including organic manures and various inorganic fertilizers such as ammonium and nitrate salts, urea, and anhydrous ammonia. Critically the management practices for the type and time of fertilizer application should minimize volatile or leaching losses (Matson *et al*., 1998). Nitrification is the conversion of ammonia into nitrate, the latter having a higher propensity for leaching from agricultural soils. Much of the N applied worldwide is in the form of ammonium and slowing the conversion to nitrate may facilitate more efficient capture (Hawkesford, 2014). Ammonium may be a better source of N for the plant as less energy is required for assimilation. Chemical nitrification inhibitors are available, but are costly. Some plant species produce exudates which are nitrification inhibitors and the transfer of this trait more widely has been proposed as a mean of improving NUE (Subbarao *et al*., 2013). It has been shown that application of N fertilizers increases the productivity of peppermint.

There is a lack of information on the influence of nitrogen fertilizer sources on peppermint phytochemical and agronomic responses. Therefore, the purpose of this research was to evaluate the biochemical and agronomy response of peppermint to different rates of nitrogen fertilizer form applied, grown in semi-arid tropical climate (Ahvaz station).

2. Materials and Methods

2.1. Experimental site preparation

The field experiment was conducted during 2015, at the Research Station, Faculty of Agronomy, Ramin Agriculture & Natural Resource University of Khuzestan, Iran (31°35′ 7.67″ N, 48° 53′ 0.81″ E, elevation 25 m above sea levels) in a semi-arid tropical climate. Before conducting the experiment, soil samples were taken from the depth of 0–30 cm to determine the physicochemical characteristics of the experimental site. Soil information is presented in Table 1.

2.2. Experimental layout and treatments

The experiment was laid out in a factorial randomized block design with three replications. There were conventional fertilization strategy including four levels of nitrogen (70, 140, 210, 280 kg N/ha) of three nitrogen fertilizer sources (urea, ammonium sulfate, urea ammonium nitrate (UAN)) and control (no added fertilizer). The intent was to use a wide enough range of nitrogen to exceed that for maximum plant requirements. N was applied in four equal splits in 15 days interval in the form of urea, ammonium sulfate and nitrate liquid. P_2O_5, in the form of triple superphosphate at the rate of 200 kg/ha was applied in each plot of the experiment. Peppermint (*Menthapiperita*) was planted on 10th February 2015 in field plots at a spacing of 30 cm. Weekly irrigation was applied, depending upon the climatic conditions. Weeds were removed by hand and farms was kept weed free during the experiment.

2.3. Plant measurements

The crop was harvested 5 cm above the ground level during the budding -flowering stage and fresh yields was recorded from an area of 1 m^2. Plant material was dried at 65°C until dry mass remained constant and biomass determined. Then leaf dry yield and essential oil yield of peppermint were recorded. The oil concentration in the air-dried herbage was estimated using Clevenger's apparatus (Mitchell and Farris, 1996).

2.4. Essential oil and GC-MS analysis

Essential oil from *Menthapiperita* was analyzed using GC/MS. The GC/MS analysis was performed on an Agilent (MS 5875 C, GC7890) system. The capillary column used was a HP-5MS (30 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 50°C, held for 5 min then raised up to 240°C at a rate of 3°C/min, then 240- 300°C at a rate of 15°C/min. Helium was used as the carrier gas at a flow rate 1 ml/min. The compounds of the oil were identified by comparison of their retention indices (RI), with data published in the literature (Adams, 2004).

2.5. Statistical analysis

The data were subjected to statistical analysis following analysis of variance (ANOVA) and regression technique as applied to factorial randomized block design (Snedecor and Cochran, 1989).

3. Results and discussion

3.1 Agronomic response of peppermint oil

Analysis of variance revealed significant differences between the nitrogen form and rates based on the fresh yield, dry yield and oil concentration of peppermint (Table 2). Maximum fresh and dry biomass yield obtained by 210 and 280 kgN/ha, while UAN exhibited better results than urea and ammonium sulfate. In control (no added fertilizer), fresh and dry yield biomass were 1150 and 379 g/m^2, respectively, but increased by adding 210 kg of nitrogen (4238, 4041, 3523 g/m^2fresh yield and 986, 982, 797g/m^2 dry yield for UAN, urea and ammonium sulfate, respectively). Result showed that all three forms of nitrogen fertilizer increased yield quantity (Figure 1a and b).Nitrogen is one of the most important modifiers of peppermint productivity and oil composition. Generally, higher N application rates were associated with high biomass yields.

Peppermint essential oil percentage in control (no added fertilizer) was 1.52 percent, while the use of 210 kgN/ha from UAN, urea and ammonium sulfate increased peppermint essential oil to 3.11, 2.65 and 2.19%, respectively. The use of more than 210 kg/ha nitrogen fertilizer led to leaf essential oil reduction (2.65, 2.36 and 1.74 by the source of UAN, urea and ammonium sulfate, respectively). The maximum percentage of essential oil obtained from 210 kg of UAN fertilizer (Figure 2).

Table 2. Analysis of variance (mean squares) of peppermint characteristics at different nitrogen rates and fertilizer form.

Source of variation	df	Fresh yield(g/m^2)	Dry yield(g/m^2)	Essential oil (%)
Block	2	3573652	210634	0.7115
N level	3	6672750**	285972**	0.7613**
N sources	2	214320^{ns}	7334^{ns}	1.164**
N level × N sources	6	302546*	30006*	0.124*
Error	22	98394	8196	0.03604
CV%	-	9.4	11.3	8.3

ns = not significant., * = P<0.05, ** = P<0.01.

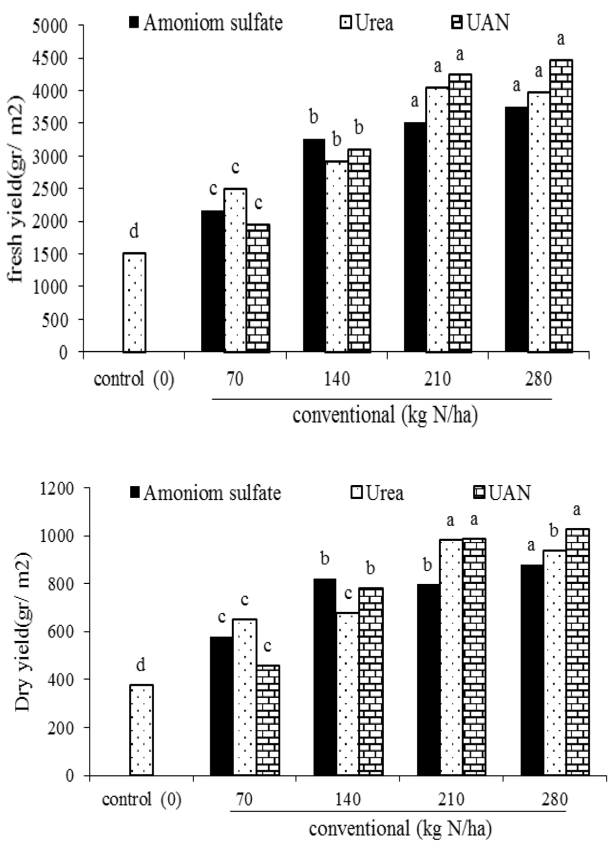

Fig. 1. Fresh (a) and dry (b) yield (g/m^2) of peppermints in control and samples with conventional fertilization.

In literatures it was reported that the most effect on peppermint yield was achieved first by the level of nitrogen and second by the level of calcium (Hornok, 1983). Saxena *et al.* (1995) reported the higher essential oil yield for corn mint, peppermint and spearmint with higher nitrogen application.

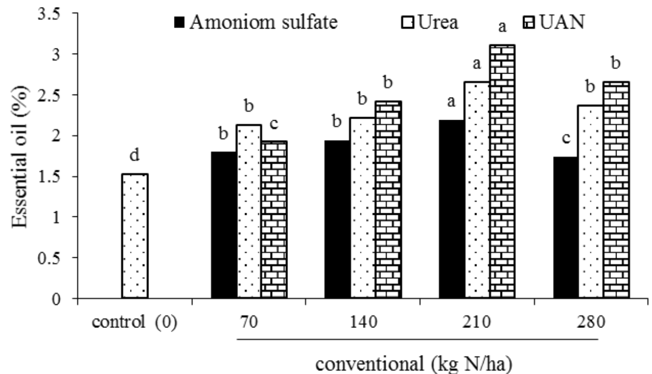

Fig. 2. Essential oil (%) of peppermints in control and samples with conventional fertilization.

3.2 Chemical composition of peppermint oil

Essential oil compounds in control and samples with 210 kg/ha of nitrogen intake was evaluated. Thirty five components were identified in peppermint oil accounting for 99% of total oil (Table 3). The essential oils from four samples (Control, 210 kg/ha UAN, urea and ammonium sulfate) were characterized according to what is listed below, respectively.

Menthol (41.22, 33.17, 30.62 and 33.23%), menthone (16.32, 26.25, 27.33 and 26.7 %), menthofuran (4.09, 4.74, 5.81 and 5.14%) and isomenthone (6.47, 7.48, 7.26 and 7.43%) were the main components of peppermint oil followed by ciscarvone oxide (8.99, 5.43, 5.74 and 5.57 %), pulegone (2.5, 2.76, 3.61 and 2.95 %), 1,8 cineole (2.71,5.79, 4.61 and 5.22%), and limonene (0.96, 1.47, 1.33 and 1.41%), trans piperitolacetatte (4.49, 3.03, 3.48 and 2.83%) and D-germacrene (3.64, 2.23, 2.5 and 2.17 %). Many studies reported the menthol as the main component of peppermint oil (Behnam *et al.*, 2006; Hawkesford, 2014; Hornok, 1983; Singh *et al.*, 1988), menthone and limonene as the second component of peppermint oil (Iscan *et al.*, 2002; Hussain *et al.*, 2010), but some others reported the β-terpinene and piperitone oxide as the main components of peppermint oil (Yadegarinia *et al.*, 2006).

Table 1. Soil physical and chemical properties of experimental area.

Treatments	Soil texture	Total N(%)	Organic Carbon(%)	E.C(dS/m)	pH	Available K (mg kgG[1])	Available P (mg kgG[1])
0-30	Silty clay	0.079	0.71	2.23	7.5	6.27	3.07

Table 3. Chemical composition (%) of the essential oils of *menthapiperita* samples

No.	Compound	KI	Control peppermint (No added fertilizer)	Conventional peppermint (Chemical fertilizers at 210 kg/ha nitrogen)		
				UAN	Urea	Ammonium sulfate
1.	α-pinene	939	0.08	0.51	0.45	0.5
2.	sabinene	975	0.23	0.44	0.4	0.41
3.	β-pinene	979	-	0.86	0.77	0.82
4.	myrcene	991	0.11	0.13	0.12	0.13
5.	octanol 3-	991	-	0.12	0.1	0.08
6.	limonene	1029	0.96	1.47	1.33	1.41
7.	1,8-Cineole	1031	2.71	5.79	4.61	5.22
8.	β-ocimene (z)	1037	0.11	0.16	0.15	0.13
9.	sabinene hydrate cis	1070	0.61	0.75	0.79	0.76
10.	trans-sabinene hydrate	1090	0.06	-	0.24	-
11.	linalool	1097	0.25	0.24	0.24	0.22
12.	isopulegole	1148	0.11	0.11	0.12	0.11
13.	menthone	1153	16.32	26.25	27.33	26.7
14.	menthofuran	1164	4.09	4.74	5.81	5.14
15.	menthoneiso	1163	6.47	7.48	7.26	7.43
16.	menthol	1172	41.22	33.7	30.62	33.23
17.	terpinen-4-ol	1177	0.35	-	0.68	0.63
18.	neo iso menthol	1187	0.73	0.42	0.36	0.42
19.	Iso- menthol	1183	0.15	0.1	0.08	0.1
20.	α-terpieol	1189	0.66	0.51	0.53	0.53
21.	pulegone	1237	2.5	2.76	3.61	2.95
22.	piperitone	1253	0.41	0.41	0.44	0.43
23.	Trans- piperiton epoxide	1254	0.45	0.29	0.3	0.31
24.	Cis- carvone oxide	1263	8.99	5.43	5.74	5.57
25.	Trans- carvone oxide	1276	0.33	0.15	0.15	0.16
26.	limonene 10 ol	1290	0.29	0.13	0.17	0.12
27.	Cis-piperitolacetatte	1335	0.31	0.14	0.19	0.13
28.	Trans- piperitolacetatte	1346	4.49	3.03	3.48	2.83
29.	α-humulene	1455	0.23	0.13	-	0.13
30.	β-farnesene	1457	0.51	0.33	0.39	0.32
31.	germacrene d	1485	3.64	2.23	2.5	2.17
32.	germacrene A	1509	0.49	0.24	0.28	0.26
33.	germacrene B	1561	0.38	0.2	0.23	0.18
34.	germacrene D 4 ol	1576	0.88	0.43	0.46	0.39
35.	β-elemenonecis	1590	0.17	-	-	-

Therefore, menthol is not always the primary component of peppermint oil. The chemical composition of peppermint oil from this study is comparable to Iscan *et al.* (2002). Menthol and menthone are the main components of the oil. Totally menthone, menthofuran, isomenthone, pulegone, 1,8 cineole and limonene increased with increasing in nitrogen fertilizer, but menthol, ciscarvone oxide and trans piperitol acetate and D-germacrene showed the reverse trend. Researchers have reported that a decrease in menthol and an increase in menthone and menthyl acetate in peppermint and corn mint has been found with increasing in nitrogen level (Duhan *et al*., 1977; Hornok, 1983). According to Kothari & Singh (1995), carvone and limonene concentrations in Scotch spearmint (*M. gracilis*) were affected by levels of N, and in general, an increase of N caused a decrease in carvone content but an increase in limonene content in essential oil. This is not quite in agreement with Singh & Singh (1986) who reported that the application of N increased carvone in spearmint oil.

4. Conclusion

Quality of peppermint essential oil was close in samples treated by chemical sources of nitrogen fertilizer including UAN and urea while it was much higher in control (no added fertilizer) treatment. Quality and quantity of essential oil in plots which were fertilized by UAN was higher than urea and ammonium sulfate. The qualitative composition of the components appeared to be constant in the three different nitrogen sources and no remarkable variation was found in the amounts of the essential oil major constituents.

5. References

Adams, R.P. 2004. *Identification of Essential Oil Components by Gas-Chromatography/Mass Spectroscopy*. Allured Publishing, Carol Stream, USA, 456p.

Behnam, S., Farzaneh, M., Ahmadzadeh, M. and Tehrani, A.S. 2006. Composition and antifungal activity of essential oils of *Menthapiperita* and *Lavendulaangustifolia* on post-harvest phytopathogens. *Communications in Agricultural and Applied Biological Sciences*., 71(3): 1321-1326.

Chalchat, J.C., Garry, R.P., Michet, A. 1997. Variation of the chemical composition of essential oil of *Menthapiperita* L. during the growing time. *J. Essent. Res.*, 9: 463–465.

Chand, S., Patra, N.K., Anwar, M., Patra, D.D. 2004. Agronomy and uses of menthol mint (*Menthaarvensis*) Indian prospective. Proc. *Indian Nat. Sci. AcadB.*, 70 (3): 269–297.

Dangour, A. D., Dodhia, S. K., Hayter, A., Allen, E., Lock, K., Uauy, R. 2009.Nutritional quality of organic foods: a systematic review. *Am. J. Clin. Nutr.*, 90: 680−685.

Duhan, SPS., Bhattacharya, AK., and Husain A. 1977. Effect of N and its methods of application on the herb and quality of corn mint. *Indian Perfume*., 21: 135–138.

Grinder-Pedersen, L., Rasmussen, S. E., Gel, S. B., Jorgensen, L. V., Dragsted, L. O., Gundersen, V., Sandstrom, B. 2003. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *J. Agric. Food Chem*., 51: 5671−5676

Hawkesford. M. J. 2014. Reducing the reliance on nitrogen fertilizer for wheat production. *Journal of Cereal Science*., 59: 276e283

Hornok L. 1983. Influence of nutrition on the yield and content of active compounds in some essential oil plants. *Acta Horticulturae*., 132: 239–247.

Hussain, A.I., Anwar, F., Nigam, P.S., Ashraf, M. and Gilani, A.H. 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*., 90: 1827-1836.

Iscan, G., Kirimer, N., kurkcouglo M., Baser K.H. and Demirci, F. 2002. Antimicrobial Screening of *Menthapiperita* Essential Oils. *Journal of Agricultural and Food Chemistry*., 50: 3943-3946.

Khanuja, S.P.S. 2007. Aroma vision 2020: Technology path of cultivating today for a fragrant tomorrow. National convention & seminar on business enabling of aromatic plants & products at Dehradun, India. November., 21-22.

Kothari SK & Singh UB. 1995. The effect of row spacing and nitrogen fertilization on scotch spearmint (*Menthagracilis* Sole). *Journal of Essential Oil Research*., 7: 287–297.

Marotti. M., R., Piccaglia. W., Crout. K., Craufutd. And S. Deans. 2004. Effect of planting time and mineral fertilization on peppermint (*Menthapiperita* L.) essential oil composition and its biological activity. *Flavor and Fragrance Journal*., 9(3): 125-129.

Matson, P.A., Naylor, R., Ortiz-Monasterio, I. 1998. Integration of environmental, agronomic, and economic aspects of fertilizer management. *Science.*, 280: 112-115.

Mitchell, A.R. and N.A. Farris. 1996. Peppermint response to nitrogen fertilizer in an arid climate. *J. Plant Nutr.*, 19: 955–967.

Murray, MJ., Marble, P., Lincoln, D & Hefendehl, FW. 1988. Peppermint oil quality differences and the reasons for them. Flavors and Fragrances: Proceeding of the 10th International Congress of Essential oils, Fragrances and Flavors, Washington, DC, U.S.A. p 189– 208.

Patra, N.K., Kumar, B., Shukla, K., Ram, P. and Srivastava, H.K. 2002. *Problems and issues of Agrotechnology transfer in menthol mint: A case study with variety Kosi*. Proceedings of First National Interactive Meet on Medicinal & Aromatic Plants CIMAP, Lucknow. pp: 440-443.

Saxena, A. and Singh, J.N. 1995. *Effect of irrigation, mulch and nitrogen on yield and composition of corn mint (Menthaarvensis L. subsp. Haplocalyx var. piperascens) oil*. Blackwell Wissenschafts-Verlag - Berlin ISSN. 0931-2250, 183–188.

Singh, A.K., Naqvi, A.A, Singh, K. and Thakur, R.S. 1988. Transformation of menthol, menthone and menthyl acetate in cornmint with relation to age of plant. *Current Science*., 57: 480-481.

Snedecor, G.W., Cochran, W.G. 1989. *Statistical Methods*, 8th ed. Iowa State University Press, Ames, Iowa.

Subbarao, G.V., Sahrawat, K.L., Nakahara, K., Rao, I.M., Ishitani, M., Hash, C.T., Kishii, M., Bonnett, D.G., Berry, W.L., Lata, J.C. 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). Ann. Bot. 112, 297-316.

Verma, R.S., Rahman L., Verma, R.K., Chauhan, A., Yadav, A.K. and Singh, A. 2010. Essential Oil Composition of Menthol Mint (*Menthaarvensis*) and Peppermint (*Menthapiperita*) Cultivars at Different Stages of Plant Growth from Kumaon Region of Western Himalaya. Journal of Medicinal and Aromatic Plants. 1(1): 13-18

Yadegarinia, D., Gachkar, L., Rezaei, MB.,Taghizadeh, M., AlipoorAstaneh, S.H. and Rasooli, I. 2006. Biochemical activities of Iranian *Menthapiperita* L. and *Myrtuscommunis* L. essential oils. *Phytochemistry*., 67: 1249- 1255.