Genetic Variation in Essential Oil Constituents of Ajwain (Trachyspermum ammi L. Sprague) Varieties at Varying Nitrogen Levels under Semiarid Tropics of Northern Karnataka, India

Article *in* Journal of Essential Oil Bearing Plants · November 2020

DOI: 10.1080/0972060X.2020.1871075

8 authors, including:

Honnappa Asangi
Indian Institute of Spices Research

MUKUND. SHRIPADRAO KULKARNI
University of Horticultural Sciences

Anand Basavanni Mastiholi
University of Horticultural Sciences

Jameel Jhalegar
University of Horticultural Sciences

Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/teop20

Genetic Variation in Essential Oil Constituents of Ajwain (*Trachyspermum ammi* L. Sprague) Varieties at Varying Nitrogen Levels under Semiarid Tropics of Northern Karnataka, India

H. Asangi , S.N. Saxena , K.N. Kattimani , M.S. Kulkarni , Y.K. Kotikal , A.B. Mastiholi , M.D. Jameel Jhalegar & R. Siddappa

To cite this article: H. Asangi , S.N. Saxena , K.N. Kattimani , M.S. Kulkarni , Y.K. Kotikal , A.B. Mastiholi , M.D. Jameel Jhalegar & R. Siddappa (2020) Genetic Variation in Essential Oil Constituents of Ajwain (*Trachyspermum ammi* L. Sprague) Varieties at Varying Nitrogen Levels under Semiarid Tropics of Northern Karnataka, India, Journal of Essential Oil Bearing Plants, 23:6, 1324-1333

To link to this article: https://doi.org/10.1080/0972060X.2020.1871075

Published online: 12 Jan 2021.

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Genetic Variation in Essential Oil Constituents of Ajwain (*Trachyspermum ammi* L. Sprague) Varieties at Varying Nitrogen Levels under Semiarid Tropics of Northern Karnataka, India

H. Asangi [1], S.N. Saxena [2] *, K.N. Kattimani [3], M.S. Kulkarni [1], Y.K. Kotikal [1], A.B. Mastiholi [1], M.D. Jameel Jhalegar [1] and R. Siddappa [1]

[1] University of Horticultural Sciences, Bagalkot, Karnataka, India
[2] ICAR-National Research Centre on Seed Spices, Ajmer, India
[3] University of Agricultural Sciences, Raichur, Karnataka, India

Received 17 June 2020; accepted in revised form 18 December 2020

Abstract: Essential oil of five varieties of ajwain was extracted and their constituents were analyzed by GC-MS. All the varieties showed a significant difference in essential oil content which ranged from 4.17 % in variety Ajmer Ajwain-1 to 5.17 % in Local cultivar. In all the varieties five major compounds including thymol, cymene, γ-terpinene, α-pinene, and β-pinene were detected. Thymol was the most abundant compound in all the varieties, the content of which varied from 53.79 % in variety Ajmer Ajwain-93 to 67.79 % in variety Ajmer Ajwain-1. The γ-terpinene content was the second most important constituent recorded 25.39 % in variety Ajmer Ajwain-93. Cymene was the other important content that showed large variation from 14.77 % in variety Ajmer Ajwain-1 to 21.61 % in Local cultivar. α-Pinene and β-pinene were other constituents that showed significant variation in varieties. Studied varieties showed significant genetic variation in essential oil constituents. However, no significant effect on essential oil constituents was observed due to varying levels of nitrogen.

Key words: Thymol, essential oil, genetic variation, nitrogen levels, *Trachyspermum ammi*.

Introduction

The seed spices have been known for ages as effective therapeutic foods. The capacity of spices to impart biological activity is now slowly reemerging as an area of interest for human health. The seed spices are mostly used in pulverized form, primarily for seasoning or garnishing food and beverages. They are characterized by pungency, strong odour, sweet or bitter taste. Among all the seed spices being used in human life, ajwain is an important and potential seed spice. Ajwain (*Trachyspermum ammi* L. Sprague) is also known as Bishop's weed or Carom seed, a member of the family *Apiaceae* is said to have originated from Eastern Mediterranean country, Egypt. It is widely distributed and cultivated in various countries such as Iran, Egypt, Pakistan, Afghanistan, and India as well as in Europe. In India, ajwain is mainly grown in the states of Rajasthan, Gujarat, Andhra Pradesh, Madhya Pradesh, Maharashtra, and Uttar Pradesh and is also cultivated in Tamil Nadu, Bihar, West Bengal, and Karnataka on small scale.

Ajwain seed contains 2.5 to 5.0 percent volatile oil which is used in many ayurvedic medi-cines and industries [1]. The oil is mainly carminative and antiflatulent. Traditionally, the seeds have been used in India as a folk remedy for arthritis, asthma, coughs, diarrhea, indigestion, intestinal gas,

*Corresponding author (S.N. Saxena)
E-mail: < shail.nrcss@gmail.com >

influenza, and rheumatism [2]. In addition to volatile oil it contains moisture (8.9 %), protein (15.4 %), fat or ether extract (18.1 %), fiber (11.9 %), carbohydrates (38.6 %) and minerals (7.1 %) [3]. Apart from these major components, major essential oil constituents thymol is well known in commercial and pharmacological approaches. The thymol content makes ajwain a potent fungicide. Thymol is also a powerful antiseptic and has an agreeable odour. Hence, it is useful in controlling a variety of fungal infections of the skin [3]. The methanolic extracts of ajwain seed possess natural antioxidant properties [4,5]. The aqueous portion left after the separation of essential oil from ajwain is known as omum-water (Ajwain water), which is used against flatulence and gripe water preparation for children [6]. Many investigations have explored the antimicrobial and antioxidant properties of this component [4,7-9]. Thymol also possesses anti-inflammatory [10], analgesic, and local anesthetic [11] effects. Moreover, it is reported that thymol can be applied for the inhibition of food-borne pathogens [12]. In addition to thymol, other major constituents of Ajwain, para-cymene, and γ-terpinene have shown beneficial effects in clinical and nutritional approaches similar to those of thymol. Although less evaluation has been done on these components, properties such as anti-inflammatory and analgesic [13] antioxidant [14] antimicrobial and inhibition of LDL oxidation [15-16] are considerable.

Though ajwain can be grown in diverse climatic conditions from arid to semi-arid performance of varieties largely depends upon nutrition and prevailing atmospheric conditions. In the present investigation, 4 ajwain varieties and a local cultivar were evaluated for two consecutive years (2016 and 2017) at semi-arid tropics of Northern Karnataka for seed essential oil yield and its constituents as a measure of seed quality. The effect of nitrogen levels was also evaluated to assess its influence on essential oil content and its major constituents.

Material and methods

An experiment was conducted for two consecutive years (2016 and 2017) during *Kharif* season in a split-plot design with three replications at the University of Horticultural Sciences, Bagalkot with 75° 42' East longitude and 16° 10' North latitude at an altitude of 542.00 m above Mean Sea Level (MSL). The experiment consisted of the main factor as five ajwain varieties namely Ajmer Ajwain-1, Ajmer Ajwain-93, Lam selection-1, and Lam selection-2 and local cultivar along with sub-plots consisting of nitrogen levels at 50 kg, 75 kg, 100 kg, and 125 kg of nitrogen per hectare.

Plant material

Five hundred grams of seeds of selected ajwain varieties were obtained from National Research Centre on Seed Spices, Ajmer (Rajasthan), and a Local cultivar were collected from farmers of Bagalkot district, Karnataka. Plot size and spacing were kept 3 x 3 m (9 sq. m) and 60 x 20 cm, respectively. The recommended dose of P_2O_5 and K_2O (50 kg ha^{-1} each) was applied to all treatments while nitrogen levels were kept 50, 75, 100, and 125 kg per hectare.

Chemicals and reagents

All chemicals and reagents (analytical HPLC grade) used in the present study were procured from Merck Co. (Germany) and Sigma-Aldrich (USA). Authentic standards of major constituents of ajwain essential oil (homologous series of C5–C24 alkanes) were procured from Sigma-Aldrich, USA.

Extraction of essential oil

Sixty grams of seeds of each variety were ground in a domestic mixer-grinder. Ground samples of each variety in three replicates were subjected to hydrodistillation for 6 hours using Clevenger apparatus [17]. After decanting and drying of the oil over anhydrous sodium sulphate the corresponding mild green coloured oil was recovered which was calculated (V/W) and the essential oil yield was expressed in percentage. The colour of essential oil remained unchanged after two hours of extraction. This essential oil was analysed to identify its constituents using GC-MS.

GC-MS analysis of essential oil

Analyses of essential oil samples were performed on an Agilent Technologies 7820A Series gas chromatography coupled to Agilent 5975 C mass selective detector. One microlitre of essential oil was mixed with hexane in the ratio 1:1000 and injected into a HP 5 MS column (Agilent, USA, 30m, 0.250 mm film thickness 0.25 µm) with the help of an autosampler (Agilent 7693). Helium was used as the carrier gas at 1.0 ml min^{-1} flow rate with a split ratio of 10:1. The oven temperature was programmed from 50°C for 3 min followed by an incremental rate at 10°C min^{-1} to 180°C and 45°C min^{-1} to 280°C. The injector and the GC-MS interface temperatures were maintained at 250°C. Mass spectra were recorded at 70 eV with a mass range from m/z 50 to 550 amu. Authentic standards of major constituents of ajwain essential oil were run alone and in combination to get the retention time of each constituent. Retention indices of all the constituents were determined by Chemstation software (Agilent Technologies, USA). All samples were analyzed in triplicate. Data were presented as means with their standard deviation (SD). Values were tested at ≤ 0.05 and were found statistically significant.

The chromatograms were analysed for constituent C_5-C_{24} compounds based on their RI (Retention Index), RT (retention time), and GC-MS library obtained on a nonpolar HP-5 MS column [18] and by comparison of mass spectra with those mentioned in NIST-MS (National Institute of Standards and Technology), a mass spectral library of the GC-MS data system and co-injection with authentic compounds. Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds.

Statistical analysis

The measured characteristics were statistically analysed and the combined analysis of test years was conducted. The software SPSS (16.1) and Microsoft Excel were used to analyse the data and draw the figures. Means were compared using the LSD test at 5 % probability level.

Results and discussion

The results indicated the significant difference in essential oil content (%) among the varieties of ajwain is presented in Fig. 1. Varieties are taken under study representing three different agro-climatic zones i.e., Western plateau and Hills, Southern plateau and hills, and Western dry region

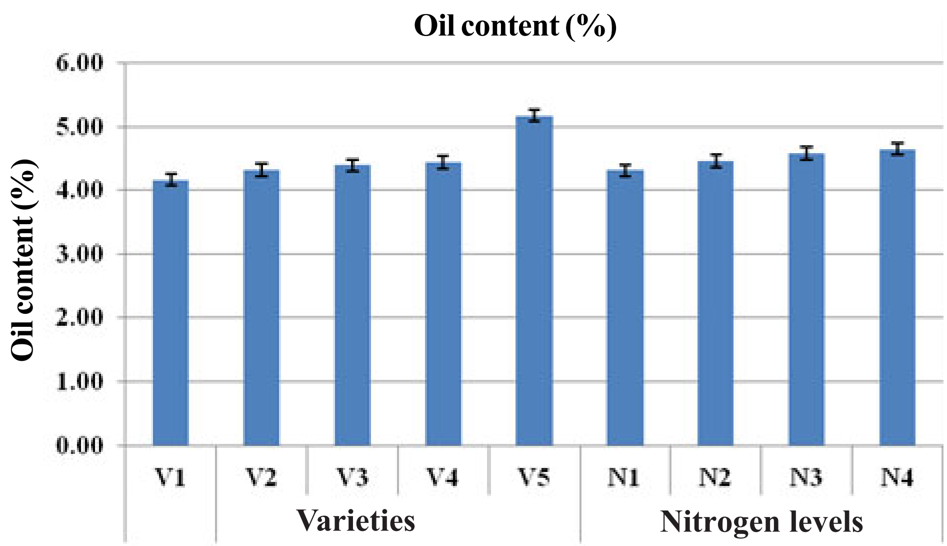

Fig. 1. Essential oil content (%) of ajwain varieties as influenced by different levels of nitrogen (pooled data)

Legend:

Main crop: Varieties (V)

V_1: Ajmer Ajwain-1
V_2: Ajmer Ajwain-93
V_3: Lam selection-1
V_4: Lam selection-2
V_5: Local cultivar

Sub plot: Nitrogen levels (N)

N_1: 50 kg N ha^{-1}
N_2: 75 kg N ha^{-1}
N_3: 100 kg N ha^{-1}
N_4: 125 kg N ha^{-1}

of India. Analysis of variance was carried out for essential oil content and major constituents (thymol, cymene, γ-terpinene, β-pinene, and α-pinene). All the varieties showed a significant difference for all the traits under study whereas nitrogen levels and interaction significantly varied for β-pinene and α-pinene (Table 1). Essential oil content was ranged from a minimum of 4.17 % in Ajmer Ajwain-1 to a maximum of 5.17 % in the local cultivar. Ajwain seeds generally contain 2.5-5 % essential oil and up to 26 % fatty oils [19-20]. The application of different levels of nitrogen did not show any significant changes in the essential oil content of seeds exept nitrogen 125 kg/ha. Production of essential oil content in seeds and other plant parts depends upon internal and external factors affecting the plant growth such as genetic structures, ecological conditions, and cultural practices have critical effects on yield and oil composition in the essential oil-bearing plants [21-24]. In the present investigation, ajwain varieties were collected from a geographical area of India representing significantly different climatic conditions as well as crop production practices. Similarly in another umbel crop like coriander Dobos and Novak [25] reported a range of variation of oil content between 0.2 and 1.3 % among 36 different coriander accessions from Austria while significant variation in essential oil content of fennel [22] and cumin [26] collected from different geographical and ecological areas of India was also reported.

Seed essential oil constituent

The data on essential oil constituents of ajwain seeds as influenced by different varieties and nitrogen levels in pooled average are presented in Table 2. The five major compounds (thymol, cymene, γ-terpinene, β-pinene, and α-pinene) were identified by GC-MS. All the varieties showed significant differences in essential oil constituents Fig. 2, 3, 4, 5, and 6). Thymol was found as the main constituent in all the varieties and found maximum (77.71 %) followed by γ-terpinene, cymene, and other constituents. Among the varieties, the highest thymol content was recorded in Ajmer Ajwain-1 (67.79 % pooled average of two years), which was *on par* with Lam selection-1 (61.58 %). This may be due to higher overall growth and yield of Ajmer Ajwain-1 among all tested ajwain varieties. The lowest thymol content was recorded in Ajmer Ajwain-93 (53.79 %) which is a short duration variety. This variation in thymol content was statistically significant (Fig. 2). However, no such effect was observed due to varying nitrogen levels on thymol content. The significance of thymol as a medicinally important phytochemical has been discussed in the introduction section of this manuscript. Earlier reports also indicated the

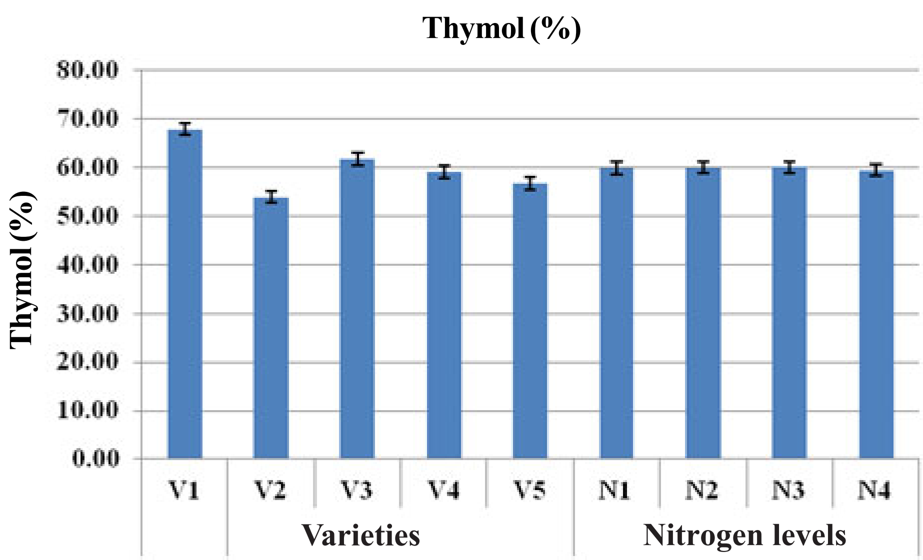

Fig. 2. Thymol content (%) of ajwain varieties as influenced by different levels of nitrogen

Table 1. ANOVA for essential oil content and major chemical constituents influenced by the varieties and nitrogen levels

SOV	df	Essential oil %	Thymol %	Cymene %	γ-Terpinene %	α-Pinene %	β-Pinene %
Replication	2	0.05	0.60	0.17	0.19	0.00003	0.003
Varieties	4	1.84* (24.8)	341.85* (51.9)	86.08* (165.8)	143.08* (236.8)	0.0335* (1096.40)	0.995* (401.0)
Nitrogen	3	0.33* (5.49)	1.03 (0.13)	0.63 (0.84)	0.26 (0.27)	0.0003* (7.30)	0.114* (29.64)
Varieties x Nitrogen	12	0.03 (0.48)	0.90 (0.12)	0.36 (0.49)	0.42 (0.44)	0.00081* (15.03)	0.065* (16.82)
Error	30	0.06	7.72	0.74	0.94	0.0005	0.004

*Significant @ 5%
Value in parenthesis is f-value

occurrence of thymol as a major constituent in ajwain seed imparting up to 50 % of total essential oil [27].

After the thymol, cymene was another important constituent identified in ajwain seeds essential oil showed significant genotypic variation. The oil obtained from the Local cultivar had maximum cymene content (21.61 %) in a pooled average of two years. While the lowest cymene content (14.77 %) was recorded in Ajmer Ajwain-1 (Fig. 3). Similar to the thymol, cymene content was also not influenced by varying levels of nitrogen. γ-Terpinene was another constituent that showed variation among the tested varieties ranged from a minimum of 16.11 % in variety Ajmer Ajwain-1 to a maximum of 25.39 % in variety Ajmer Ajwain-93. Varying nitrogen levels did not result in any significant change in γ-terpinene content (Fig. 4). Similar to the cymene content, β-pinene was also found maximum in Local variety and minimum in Ajmer Ajwain-1 (Fig. 5). α-Pinene was found less than 1.0 % in all the varieties and different nitrogen levels (Fig. 6).

Many factors including genetic and environmental are responsible for the synthesis of essential oil and bioconversion of its constituents. In the present study also the effect of weather conditions made a significant impact on essential oil content as well as its constituents. A perusal of weather data of two years (Table 3) revealed more rains during 2017 and a little bit more minimum temperature, as a result, showed higher thymol content as compared to *Kharif* 2016. However, γ-terpinene and cymene were more during 2016. This change is realized with the fact that the synthesis of thymol and cymene is taking place by bioconversion of γ-terpinene [28]. The varieties that showed higher thymol content showed less terpinene and cymeme and vice-versa.

It is well documented that genetic constitution and environmental conditions influence the yield and composition of volatile oil produced by medicinal plants [21-24,26,29]. Unlike other yield attributing characters that are quantitatively inherited and highly affected by the environment, essential oil composition depends upon internal and external factors affecting the plant growth such

Table 2. GC-MS analysis of identified constituents in different genotypes of ajwain essential oil (Pooled average of two years)

Major EO constituents	α-Pinene	β-Pinene	Myrecene	Cymene	γ-Terpinene	4-allyl anisole	Geraniol	Thymole	Geranyl Acetate
RT	5.348	6.017	6.17	6.762	7.297	9.399	10.144	10.832	11.654
RI	948	943	958	979	998	1172	1228	1262	1352
Identification	RI, MS	RI, MS	RI, MS	RI, MS, Co GC	RI, MS, Co GC	MS	RI, MS, Co GC	RI, MS	RI, MS, Co GC
V_1N_1	0.04	0.85	0.08	15.06	16.07	0.08	0.00	67.04	0.06
V_1N_2	0.07	0.91	0.17	14.15	16.05	0.03	0.01	68.73	0.02
V_1N_3	0.07	0.75	0.19	14.44	15.97	0.22	0.00	68.46	0.01
V_1N_4	0.07	0.82	0.25	15.43	16.34	0.05	0.01	66.93	0.03
V_2N_1	0.11	1.39	0.45	18.71	24.78	0.07	0.04	54.13	0.05
V_2N_2	0.13	1.23	0.31	18.97	25.44	0.05	0.01	53.80	0.02
V_2N_3	0.14	1.30	0.28	18.53	25.88	0.28	0.02	53.63	0.06
V_2N_4	0.12	1.33	0.37	18.92	25.45	0.01	0.02	53.60	0.03
V_3N_1	0.11	1.26	0.27	15.96	20.12	0.16	0.01	61.95	0.02
V_3N_2	0.14	1.25	0.38	16.62	20.04	0.01	0.00	60.79	0.01
V_3N_3	0.12	1.27	0.34	15.77	19.93	0.19	0.01	62.14	0.03
V_3N_4	0.13	1.24	0.32	15.64	20.69	0.12	0.01	61.43	0.02
V_4N_1	0.13	1.41	0.33	16.74	21.59	0.20	0.01	59.06	0.03
V_4N_2	0.09	0.90	0.22	16.87	21.55	0.04	0.01	59.54	0.01
V_4N_3	0.13	1.40	0.35	16.56	22.54	0.01	0.01	58.74	0.02
V_4N_4	0.13	1.41	0.40	17.14	21.77	0.04	0.00	58.73	0.02
V_5N_1	0.23	1.66	0.41	21.57	19.01	0.05	0.03	56.79	0.02
V_5N_2	0.19	1.49	0.41	21.67	19.26	0.04	0.02	56.60	0.03
V_5N_3	0.21	1.47	0.37	21.30	18.60	0.27	0.01	56.82	0.02
V_5N_4	0.21	1.95	0.57	21.93	18.75	0.01	0.01	56.18	0.01

MS-Mass spectrum
CoGc-Co- injection with authentic compounds
RT-Retention time and RI- Retention index

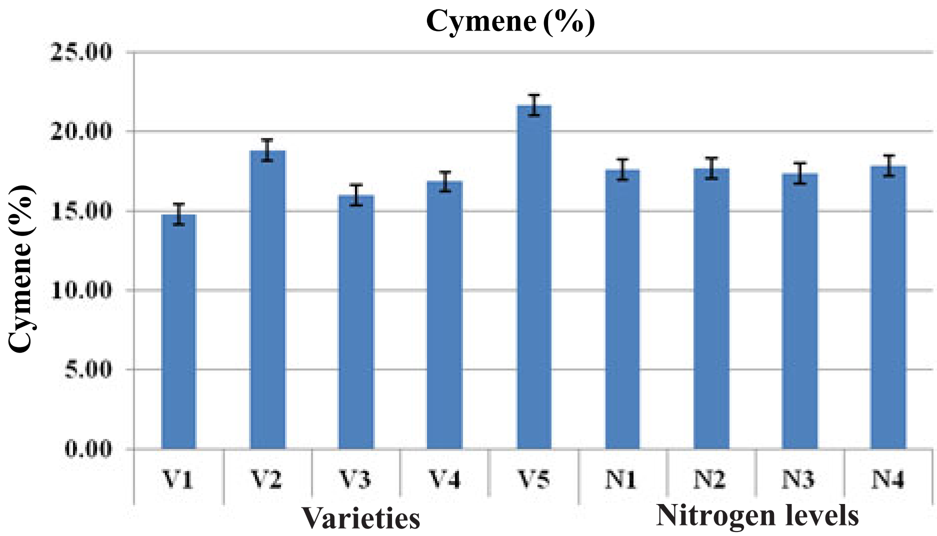

Fig. 3. Cymene content (%) of ajwain varieties as influenced by different levels of nitrogen

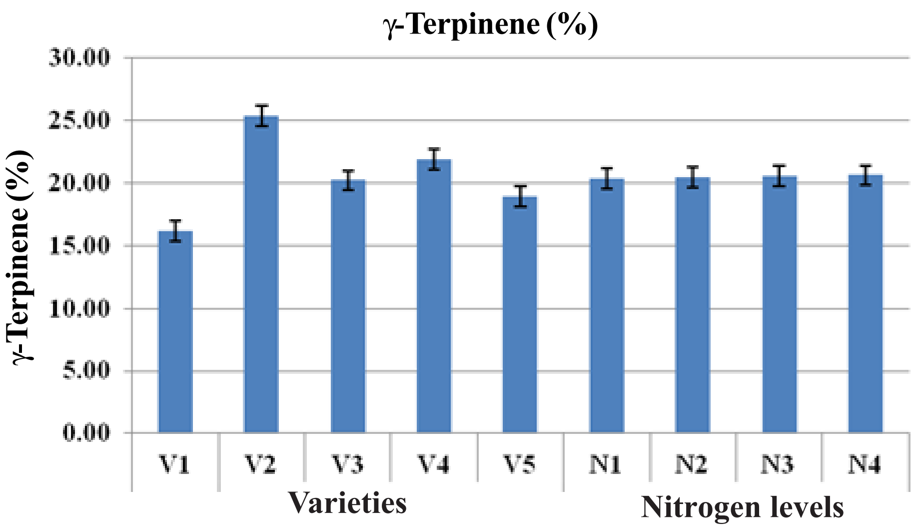

Fig. 4. γ-Terpinene content (%) of ajwain varieties as influenced by different levels of nitrogen

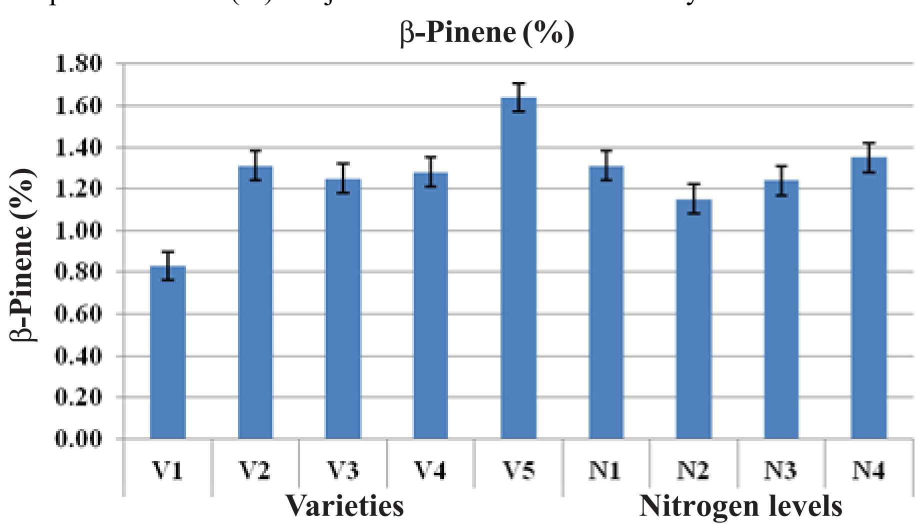

Fig. 5. β-Pinene (%) of ajwain varieties as influenced by different levels of nitrogen

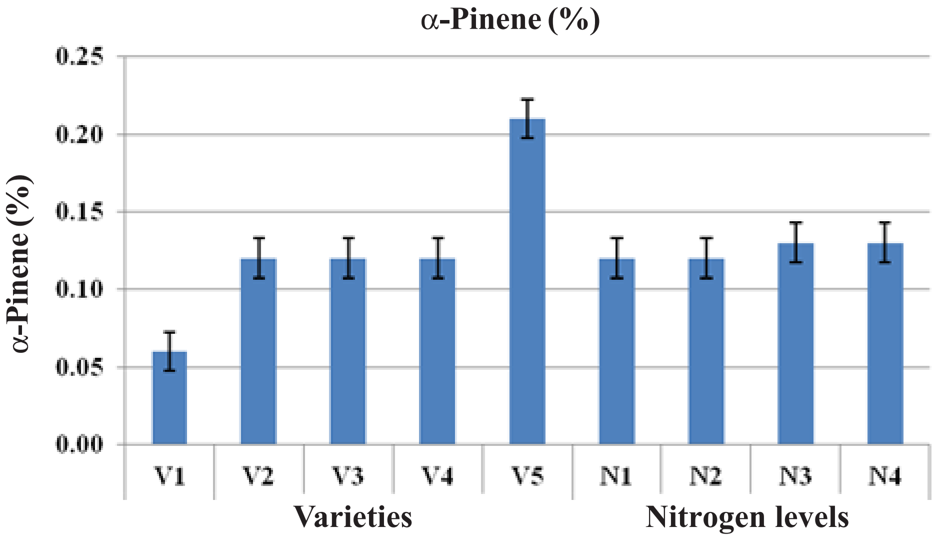

Fig. 6. Alpha Pinene (%) of ajwain varieties as influenced by different levels of nitrogen

Table 3. Meteorological data recorded during the experimental period (2016 and 2017) at the Main Horticultural Research and Extension Centre, University of Horticultural Sciences, Bagalkot

Months	Mean temperature (°C) Maximum		Minimum		Relative humidity (%)		Rainfall (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017
January	30.30	21.47	12.30	14.00	66.00	46.00	0.00	0.00
February	35.90	31.40	16.20	17. 00	70.00	43.00	0.00	2.00
March	37.60	34.80	20.70	19.00	74.00	37.00	0.00	5.00
April	37.80	35.75	21.80	23.00	76.00	43.00	26.60	21.00
May	37.10	31.90	22.60	23.00	76.00	55.00	41.10	51.00
June	32.10	29.00	21.60	22.00	81.00	84.00	137.40	69.00
July	29.70	28.00	20.40	21.00	87.00	89.00	61.30	64.00
August	29.50	29.00	19.90	21.00	88.00	86.00	14.60	70.00
September	27.30	28.00	18.50	21.00	88.00	85.00	71.60	139.00
October	30.60	30.90	16.90	18.00	86.00	84.00	0.00	93.00
November	30.80	28.00	14.00	17.00	18.00	70.00	0.00	29.00
December	33.00	28.40	14.00	15.20	66.00	62.00	0.00	9.00

as genetic structures and ecological conditions. The varieties taken for the study are having distinct morphology. Ajmer Ajwain-1 and Ajmer Ajwain 93 were selected from South Rajasthan but differ in seed size and crop duration. Ajmer Ajwain-1 is a long-duration variety with bold seed while Ajmer Ajwain-93 is a short-duration variety with small seeds. Lam selection-1 and 2 are developed from Southern India and comparatively short duration than Ajmer Ajwain-1. Agricultural practices also have critical effects on yield and oil composition in the essential oil-bearing plants [30].

Conclusions

In the present study, no significant effect of varying nitrogen levels and the interaction effect between varieties and nitrogen levels on quality attributes have been observed in the pooled analysis of two years of data except α- and β-pinene content. However, a significant effect of genotypes was observed indicating more genetic factors influencing intrinsic quality parameters. Ajwain can be a good source of pharmacologically important compound thymol which is highly dependent upon genotype. Having a specific chemotype with a high amount of major components may be beneficial for investigational and functional approaches in the food and pharmaceutical industries. It should be noted that these main components, thymol, cymene, and γ-terpinene, exhibit different pharmacological and nutritional properties, individually.

Acknowledgement

A field experiment was conducted at the University of Horticultural Sciences, Bagalkot, Karnataka. The authors are thankful to Director, NRCSS, Tabiji, Ajmer for providing GC-MS facilities to carry out this work.

Conflict of interest

No conflict of interest.

References

1. **The Ayurvedic Pharmacopoeia of India (2016).** Government of India, Ministry of Health and Family Welfare Department of Ayush. Part 1, Vol. 9. Pharmacopoeia Commission for Indian Medicine & Homoeopathy, PLIM Campus, Kamla Nehru Nagar, Ghaziabad-201002 (U.P.) India. pp 1-182
2. **Sayre, J.K. (2001).** Ancient Herbs and Modern Herbs. Bottlebrush Press, San Carlos, CA.
3. **Bairwa, R. (2011)**. Medicinal uses of *Trachyspermum ammi* : A review. Pharm. Res., 5: 247-258.
4. **Saxena, S.N., Agarwal, D., Saxena, R. and Rathore, S.S. (2012).** Analysis of anti-oxidant properties of ajwain (*Trachyspermum ammi* L) seed extract. Int.J. Seed Spices. 2(1): 50-55.
5. **Aeschbach, R., Löliger, J., Scott, B.C., Murcia, A., Butler, J., Halliwell, B. (1994).** Anti-oxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxicol. 32: 31-6.
6. **Prajapati, N.D., Purohit, S.S., Sharma, A. and Kumar, T. (2003).** A Handbook of Medicinal Plants. Agribios India, Jodhpur, India, pp. 362-3.
7. **Olasupo, N.A., Fitzgerald, D.J., Gasson, M.J., Narbad, A. (2003).** Activity of natural anti-microbial compounds against Escherichia coli and Salmonella enterica serovar Typhimurium. Lett. Appl. Microbiol. 37: 448-51.
8. **Sharma, L.K., Agarwal, D., Saxena, S.N., Kumar, H., Kumar, M., Verma, J.R. and Singh, B. (2018).** Antibacterial and Antifungal activity of ajwain (*Trachyspermum ammi*) in different solvent, J. Pharmacog. and Phytochem. 7(3): 2672-2674.
9. **Bagamboula, C.F., Uyttendaele, M., Debevere, J. (2004).** Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri.* Food Microbiol. 21: 33-42.
10. **Braga, P.C., Dal Sasso, M., Culici, M., Bianchi, T., Bordoni, L., Marabini, L. (2006).** Anti-inflammatory activity of thymol: Inhibitory effect on the release of human neutrophil elastase. Pharmacol. 77: 130-6.
11. **Haeseler, G., Maue, D., Grosskreutz, J., Bufler, J., Nentwig, B., Piepenbrock, S. (2002).** Voltage-dependent block of neuronal and skeletal muscle sodium channels by thymol and menthol. Eur. J. Anaesthesiol. 19: 571-9.
12. **Karapinar, M., Aktuð, E.S. (1987).** Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. Int. J. Food Microbiol. 4: 161-6.
13. **Hajhashemi, V., Ghannadi, A., Jafarabadi, H. (2004).** Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytothe. Res. 18: 195-9.
14. **Foti, M.C., Ingold, K.U. (2003).** Mechanism of Inhibition of Lipid Peroxidation by γ-Terpinene, an Unusual and Potentially Useful Hydrocarbon Antioxidant. J Agri. Food Chem. 51: 2758-65.

15. **Delgado, B., Fernández, P.S., Palop, A., Periago, P.M. (2004).** Effect of thymol and cymene on *Bacillus cereus* vegetative cells evaluated through the use of frequency distributions. Food Microbiol. 21: 327-34.
16. **Milde, J., Elstner, EF., Graßmann, J. (2004).** Synergistic inhibition of low-density lipoprotein oxidation by rutin, γ-terpinene, and ascorbic acid. Phytomedicine. 11: 105-13.
17. **Clevenger, J.F. (1928).** Apparatus for determination of essential oil. J. Am. Pharm. Assoc. 17: 346-49
18. **Adams, R.P. (2004).** Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL: Allured Publishing Corp. US
19. **Raghavan S. (2007).** Handbook of Spices, Seasoning and Flavourings, 2nd ed, CRC Press, Taylor & Francis group, Boca Raton, FL, New York., 107-119.
20. **Sharma, L.K., Agarwal, D., Meena, S.K., Rathore, S.S. and Saxena, S.N. (2015).** Effect of cryogenic grinding on oil yield, phenolics and antioxidant properties of ajwain (*Trachyspermum ammi* L.). Int. J. Seed Spices. 5(2): 82-85.
21. **Saxena, S.N., Sharma, Y.K., Rathore, S.S., Singh, K.K., Barnwal, P., Saxena, R., Upadhyaya, P. and Anwer, M.M. (2015).** Effect of cryogenic grinding on volatile oil, oleoresin content and anti-oxidant properties of coriander (*Coriandrum sativum* L.) genotypes. J. Food Sci. Technol. 52(1): 568-573
22. **Saxena, S.N., Kakani, R.K. Rathore, S.S., Meena, R.S., Vishal, M.K., Sharma, L.K., Agrawal, D., John, S., Panwar A. and Singh, B. (2016).** Genetic variation in essential oil constituents of fennel (*Foeniculum vulgare* Mill) germplasm. J. Essential Oil Bearing Plants. 19(4): 989-999
23. **Saxena, S.N., Rathore, S.S., Saxena, R., Barnwal, P., Sharma, L.K. and Singh, B. (2014).** Effect of cryogenic grinding on essential oil constituents of coriander (*Coriandrum sativum* L.) genotypes. J. Essential Oil Bearing Plants. 17(3): 385-392.
24. **Agarwal, D., Saxena, S.N., Sharma, L.K. and Lal, G. (2018).** Prevalence of essential and fatty oil constituents in fennel (*Foeniculum vulgare* Mill) genotypes grown in semi arid regions of India. J. Essential Oil Bearing Plants. 21(1): 40-51
25. **Dobos, G. and Novak, J. (2005).** Comparison of the composition of the essential oil of some winter-annually cultivated coriander accessions (*Coriandrum sativum* L.). Zeitschrift fur Arznei- & Gewurzpflanzenn 10: 144-145.
26. **Dubey, P.N., Saxena, S.N., Mishra, B.K., Solanki, R.K., Vishal, M.K., Singh, B., Sharma, L.K., John, S., Agarwal, D., and Yogi, A. (2017).** Preponderance of cumin (*Cuminum cyminum* L.) essential oil constituents across cumin growing Agro-Ecological Sub Regions, India. Ind. Crop Prod. 95: 50-59.
27. **Joshi, S.G. (2000).** Medicinal Plant, Oxford and IBH Publisher, New Delhi (India). 51-52.
28. **Poulose, A.J., Croteau, R. (1978).** Biosynthesis of aromatic monoterpenes: Conversion of gamma-terpinene to p-cymene and thymol in *Thymus vulgaris* L. Arch Biochem. Biophys. 187: 307-14.
29. **Ramezani, S., Ramezani, F., Rasouli, F., Ghasemi, M., Fotokian, M.H. (2009).** Diurnal variation of the essential oil of four medicinal plants species in central region of Iran. Res. J. Biol. Sci. 4: 103-6.
30. **Telci, I., Demirtas, I., Sahin, A. (2009).** Variation in plant properties and essential oil composition of sweet fennel (*Foeniculum vulgare* Mill.) fruits during stages of maturity. Ind. Crop. Prod. 30: 126-130.