



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 548-552
© 2019 TPI
www.thepharmajournal.com
Received: 09-11-2018
Accepted: 13-12-2018

Subramaniyan P
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

L Jeeva Jothi
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

K Sundharaiya
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

N Shoba
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

S Murugesan
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

Correspondence
Subramaniyan P
Department of Spices and
Plantation Crops, Horticultural
College and Research Institute,
Tamil Nadu Agricultural
University, Periyakulam, Tamil
Nadu, India

Extraction of essential oil and Thymol from different Ajowan (*Trachyspermum ammi* L.) genotypes using gas chromatography

Subramaniyan P, L Jeeva Jothi, K Sundharaiya, N Shoba and S Murugesan

Abstract

Ajowan (*Trachyspermum ammi* L.) is a herbaceous annual plant, belonging to the family Apiaceae. Ajowan seeds are used as a medicament in Unani, Ayurvedic and Arab traditional medicine for its diuretic, analgesic, antiasthmatic, anthelmintic and antispasmodic properties. The fruit contains an aromatic volatile essential oil, a crystalline substance stearoptene. The thymol and carvacrol derivatives and other minor components of ajowan are responsible for its functional properties. The present investigation was carried out to study the essential oil, thymol, *p*-cymene and γ -terpinene content in the different genotypes of ajowan. The functional properties were estimated by using gas chromatography at the department of Horticulture, University of Agricultural Science, GKVK, Bangalore-65. The result revealed that the highest essential oil content of 4.6 per cent was recorded by LTA- 26 4.0 per cent and it was followed by JA-176, Acc No- 5 (3.8 percent) and Acc No- (3 3.4%). The lowest essential oil content of 2.9 per cent was recorded by JA-186. The highest thymol content of 50.06 per cent was recorded by LTA-26. It was followed by Acc. No.- 3 (49.54 per cent) and GA-1 (47.44 per cent). Whereas the lowest thymol yield was recorded by Acc. No.- 5 (39.2 5per cent). The highest of 25.26 per cent was recorded by JA186., It was followed by Acc. No. 5 (23.15 per cent) and JA-110 (23.11 per cent) and the lowest *p*-cymene content was recorded by JA-176 (15.83 per cent). The highest γ -terpinene content of 31.53 per cent, 28.88 per cent and 25.69 per cent were recorded by Acc. No. – 5, JA-110 and JA-186 respectively and the lowest was observed in JA-176 (20.83 per cent).

Keywords: Ajowan, thymol, *p*-cymene and γ -terpinene

1. Introduction

Ajowan (*Trachyspermum ammi* L.) is a herbaceous annual plant, belonging to the family Apiaceae. It is a minor seed spice with medicinal value. It is also known as Bishop's weed, Carum seed or Carum, ajowan (Bentley and Trimen, 1983) [7]. Ajowan is indigenous to India and Egypt (Sayre, 2001) [27]. Ajowan is majorly cultivated in the Mediterranean region and South-West Asian countries. In India, it is cultivated in the states of Rajasthan and Gujarat in a large scale and to a limited extent in Uttar Pradesh, Bihar, Madhya Pradesh, Punjab, Tamil Nadu, West Bengal and Andhra Pradesh. India is the largest producer and exporter of the ajowan seed in the world exporting to around 46 countries (Malhotra and Vijay, 2002) [16]. Ajowan seed is used as a medicine for relief of pain in human digestive track and as an antibloat (Mirheidar, 1993) [19]. Ajowan fruits are used as a medicament in Unani, Ayurvedic and Arab traditional medicine for its diuretic, analgesic, antiasthmatic, anthelmintic and antispasmodic properties (Zahin *et al.*, 2010) [34]. The fruit contains an aromatic volatile essential oil, a crystalline substance stearoptene (crude thymol) a phenolic glucoside (Ballba *et al.*, 1973) [5]. According to Anilkumar *et al.* (2009) and Pathak *et al.* (2010) [22] the thymol and carvacrol derivatives and other minor components of ajowan are responsible for its functional properties. The seed yield and thymol content is a complex character. Therefore, the knowledge on the optimal plant characteristics, the extent of variability available and character association with seed yields is essential to improve the yield in ajowan. It is reported that ajowan oil contains thymol (39.1%) as a major component along with *p*-cymene (30.8%), γ -terpinene (23.2%), β - pinene (1.7%), terpinene-4-ol (0.8%). It contains mainly volatile chemical constituents. Essential oils are usually analyzed by gas chromatography (GC), because of its high sensitivity. It is most widely used for quick and easy determination of quality, authenticity and purity of the crude drugs and formulations by estimation of markers compound.

The objective of the present study was to develop identification, accurate, specific and reproducible method for the estimation of thymol in ajowan seeds formulation. The developed method is also utilized to determine the purity and quality of the market sample by comparing with the prepared authentic formulation.

2. Materials and Methods

The present investigation on "Extraction of essential oil and thymol from different ajowan (*Trachyspermum ammi* L.) genotypes using gas chromatography" was conducted at the department of Horticulture, University of Agricultural Science, GKVK, Bangalore- 65. The field evaluation was carried out at the Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam during August 2012 to May 2013. The experimental materials includes twenty

ajowan genotypes obtained from Tamil Nadu (6 Nos.), Gujarat (12 Nos.) and Andhra Pradesh (2 Nos.). The experiment was laid out in a randomized block design and replicated thrice. The experimental field was ploughed thrice to get fine tilth and prepared flat beds size of 7 x 1 m², spacing - 45 x 30 cm, bed size - 7 m². All the recommended cultural operations were carried out uniformly and a separate fertilizer dose at the rate of 80: 30: 30 kg of NPK ha⁻¹, 5 kg of Sulphur was applied to the field. After harvesting, Only eight ajowan genotypes were selected based on the growth and yield performance for extraction of essential oil, thymol, *p*-cymene and γ -terpinene from different ajowan (*Trachyspermum ammi* L.) genotypes using gas chromatography (Table.1). The essential oil content was estimated as per the method suggested by ASTA (Anon, 1968).

Table 1: Details of ajowan genotypes for analyzing the thymol content

S. No.	Treatment	Genotypes	Accession No.	Source
1	T3	Acc:3	Lam Selection-1	Horticultural Research Station- Lam, Guntur (AP)
2	T4	Acc:4	LTa- 26	Horticultural Research Station- Lam, Guntur (AP)
3	T5	Acc:5	Gujarat Ajowan-1	Agricultural Research Station- Jagudan, Gujarat
4	T7	Acc:7	JA-110	Agricultural Research Station- Jagudan, Gujarat
5	T9	Acc:9	JA-176	Agricultural Research Station- Jagudan, Gujarat
6	T14	Acc:14	JA-186	Agricultural Research Station- Jagudan, Gujarat
7	T17	Acc:17	Acc. No. 5	Horticultural College and Research Institute, Periyakulam
8	T18	Acc:18	Acc. No. 3	Horticultural College and Research Institute, Periyakulam

3. Result and Discussion

Morphological traits are the highest determinants of agronomic value and taxonomic classification of plants when they are compared with others for mean, morphological evaluations. However, morphological estimations are more dependent on the environment and the reliability of these measurements can be improved by the effective control of the environmental variation. In the present study, morphological traits *viz.*, plant height, number of branches, number of umbels were studied and the genotypes were evaluated based on the *per se* value (Table. 2). Identification of superior types in the available ajowan genotypes was attempted based on the morphological, physiological, yield and quality traits. Ajowan genotypes exhibited significant variations for biometrical, yield and quality characters which offer scope for selection of high yielding genotypes. Among the 20 genotypes evaluated, the Accession No.3 registered high seed yield (38.83 g) followed by LTa-26 (35.21 g), LS-1(32.61 g) and GA-1(32.54 g) (Table. 2). The yield components such as plant height, number of primary branches per plant, number of umbels per plant, number of umbellets per umbel and seed recovery per umbel were similar in high yielding genotypes as reported by Rajendran (1978) ^[24], Suthanthira Pandian *et al.* (1980) ^[31], Raja Gopalan *et al.* (1996) ^[23], Umanath (1998) ^[33], Sharma and Bhatt (1988) ^[28] in coriander, Singh *et al.* (2003) ^[30] and Garg *et al.* (2003) ^[14] in fennel.

In the present study, high yielding genotypes were characterized with early vigorous growth as evident from higher mean values for plant height, number of primary branches, number of umbels, number of umbellets and seed recovery per umbel. At the same time, low yielding genotypes were less vigorous in growth with poor expression of all the morphological characters. The present findings are in conformity with the earlier results of Mohideen (1978) ^[21] in amaranthus, Dhanasekar (1997) ^[12] in coriander and Ann Riya (2001) ^[2] in coriander. In the present study it was noticed that the genotypes performed differently for the various quantitative and qualitative traits. This has lead to the inference that independent selection of genotypes has to be exercised for yield and quality characters.

Eight selected ajowan genotypes *viz.*, Acc. No. - 3 (LS-1), Acc. No. - 4 (LTa-26), Acc. No. - 5 (GA-1), Acc. No. - 7 (JA-110), Acc. No. - 9 (JA-176), Acc. No. - 14 (JA-186), Acc. No. - 17 (Acc. No. - 5) and Acc. No. - 18 (Acc. No.3) were used for estimation of essential oil (Table-3) and major constituents *viz.*, *p*-cymene, γ -terpinene and thymol through gas chromatography. The gas chromatography revealed that the *p*-cymene content varied from 15.83 % (JA-176) to 25.26 % (JA-186). The highest *p*-cymene content of 25.26%, 23.15% and 23.11% was recorded by JA-186, Acc. No. - 5 and JA-110 (Fig. 1.f, g & d).

Table 2: *Per se* performance for growth and yield parameters of ajowan genotypes

Genotype	Plant height (cm)	Number of primary branches per plant	Number of secondary branches per plant	Number of umbels per plant	Number of flowers per umbel	Number of seeds per umbel	Seed yield per plant (g)	Seed yield per hectare (t)
Acc. No. 11	122.8	15.20	78.60	239.9	214.4	166.7	18.60	1.377
Acc. No. 13	111.1	13.53	70.40	245.2	206.6	207.3	15.13	1.120
LS- 1	109.1	14.93	78.27	293.8	267.0	259.5	32.61	2.417
LTa- 26	115.4	15.87	88.27	258.2	291.4	211.6	35.21	2.607
GA-1	125.9	14.60	80.47	297.5	318.8	296.8	32.54	2.410

JA- 106	107.3	14.67	99.60	296.3	220.0	230.3	20.92	1.547
JA-110	106.7	13.33	73.50	225.3	282.6	263.5	27.04	2.003
JA-114	99.24	13.93	62.30	253.6	217.0	217.2	20.82	1.543
JA-176	102.8	13.73	81.00	249.8	244.0	211.5	27.55	2.037
JA-177	108.0	14.40	84.60	235.7	285.0	176.8	19.26	1.430
JA-145	86.52	13.67	52.40	215.7	233.2	218.7	18.67	1.383
JA-172	99.68	14.00	80.70	288.5	184.2	213.9	27.73	2.053
JA-179	107.0	13.20	80.20	294.1	223.0	199.2	19.59	1.450
JA-186	104.0	13.33	80.87	284.9	247.6	194.3	18.33	1.357
JA-190	116.9	12.87	55.00	246.8	248.8	204.4	24.31	1.800
JA-212	110.9	12.47	63.30	238.5	264.0	226.2	22.76	1.687
Acc. No. 5	95.72	15.20	69.80	201.8	206.6	211.8	24.09	1.783
Acc. No. 3	126.0	16.80	116.1	322.6	339.6	315.0	38.83	2.807
Acc. No. 9	85.92	11.80	68.20	188.1	193.8	158.7	30.14	2.233
Acc. No. 14	100.6	15.90	104.5	148.1	188.8	209.1	24.90	1.843
Mean	107.1	14.17	78.40	251.2	243.8	219.6	24.95	1.844
S. Ed.	4.128	0.729	5.061	11.96	12.24	17.44	1.209	0.089
C.D. 5%	11.82	2.088	14.49	34.23	35.04	49.94	3.461	0.255
C.V.	6.678	8.914	11.18	8.244	8.696	13.76	8.393	8.351

The lowest p- cymene content of 15.83% was recorded by JA-176. The γ -terpinene content varied from 20.82 % (JA-176) to 31.53 % (Acc. No.-5). The highest γ -terpinene content of 31.53 %, 28.88% and 25.69% were recorded by Acc. No. – 5, JA-110 and JA-186 (Fig. 1.g, d & f). The lowest γ -terpinene content of 20.83% was recorded by JA-176. The thymol varied from 39.25% (Acc. No. - 5) to 50.06% (LTa- 26). The highest thymol content of 50.06 per cent, 49.54 per cent, and 47.44 per cent was recorded by LTa-26, Acc. No. - 3 and GA-1. The lowest thymol content of 39.25 per cent was recorded by Acc. No. – 5. The essential oil content of various genotypes of ajowan varied from 2.9-4.6 per cent, in which Acc. No. – 4(LTa-26) and Acc. No. - 9 (JA-176) showed highest oil content of 4.6 per cent (Fig.1.b& e). The visual appearance of all the oils was clear except for Acc. No. – 4(LTa-26) and Acc. No. - 17 (Acc. No. 5) which showed turbidity (Fig.1.b&g). Acc. No. - 4 (LTa-26) showed a highest percentage of thymol content (50.06%) as compared to other genotypes (Fig.1.b). Goudarzi *et al.* (2011) [15] identified twelve chemical compositions *Trachyspermum ammi* essential oil. The major constituents of *Trachyspermum ammi* were thymol and its precursors, γ - terpinene and p- cymene. Similarly, Thangam and Dhananjayan (2003) [32] in carum;

Behravan *et al.* (2007) [6] in *Zataria Multiflora*, *Carum copticum* and *Thymus vulgaris*) found thymol to be the major constituent of the oil known as 'Ajwan-kaphul' (crude thymol), while others reported carvacrol as the major constituent of this oil (Mohagheghzadeh *et al.*, 2007 in carum) [20]. Zambonelli *et al.*, (2004) [35] reported that the thymol, γ -terpinene and p-cymene were founded in ajowan. The major constituents of ajowan oils were thymol, p-cymene and γ -terpinene was reported by Raina *et al.* (2004) [25] Chialva *et al.* (1993) [8] reported that the oils of ajowan contain thymol under GC and GC- MS method.

4. Summary

An investigation was carried out in ajowan (*Trachyspermum ammi* L.) with twenty genotypes to assess the extent of growth, yield and quality parameters. Twenty genotypes of ajowan were evaluated in a randomised blocks design with three replications during 2012- 2013 for the traits *viz.*, plant height, number of primary branches, number of secondary branches, number of umbels per plant, number of seeds per umbel, seed yield per plant observed and results are summarized below.

Table 3: Estimation of essential oil content (%), p-Cymene, γ -Terpinene and thymol by gas chromatography

S. No.	Accession	Name of the génotypes	Essential oil content (%)	Area (%)		
				p-Cymene	γ -Terpinene	Thymol
1.	Acc. No. - 3	LS- 1	3.1	22.43	27.83	44.94
2.	Acc. No.- 4	LTa- 26	4.6	21.62	22.32	50.06
3.	Acc. No.- 5	GA- 1	3.4	20.56	25.58	47.44
4.	Acc. No.- 7	JA- 110	3.3	23.11	28.88	42.42
5.	Acc. No.- 9	JA- 176	4.6	15.83	20.82	44.16
6.	Acc. No.- 14	JA- 186	2.9	25.26	25.69	42.25
7.	Acc. No.- 17	Acc. No.- 5	4.0	23.15	31.53	39.25
8.	Acc. No.- 18	Acc. No.- 3	3.8	21.47	24.20	49.54

Based on the *per se* performance the genotypes Acc. No.3 (38.83g), LTa-26 (35.21g), LS-1(32.61g) and GA-1(32.54g) were identified as superior for the traits *viz.*, plant height, number of primary branches, number of secondary branches, number of umbels per plant, number of seeds per umbel and seed yield per plant. Based on the mean performance, the genotypes Acc. No.3, LTa-26, LS-1 and GA-1 recorded higher seed yield per plant which were due to the contributing parameters *viz.*, plant height, number of primary branches, number of secondary branches, number of umbels per plant, number of flowers per umbel, number of seeds per umbel and

seed yield per plant. Therefore, the above genotypes could be well exploited in future breeding programmes and selection of varieties for the respective traits. The essential oil extracted from selected ajowan genotypes were found to contain the major constituents *viz.*, p- cymene, γ -terpinene and thymol which were estimated through gas chromatography. It was found that the accessions JA-186, Acc. No. 5 and JA-110 recorded the highest p- cymene contents; Acc. No. 5, JA-110 and JA-186, the highest γ -terpinene contents; and LTa-26, Acc. No. - 3 and GA-1, the highest thymol contents.

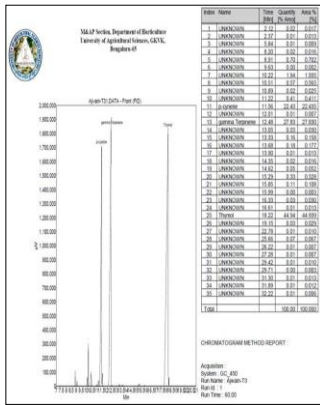


Fig 1a: ACC No. 3

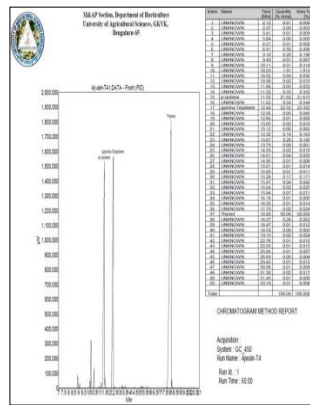


Fig 1b: ACC No. 4

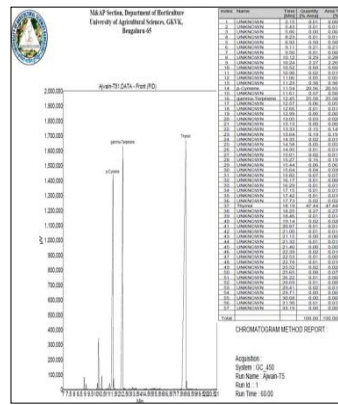


Fig 1c: ACC No. 5

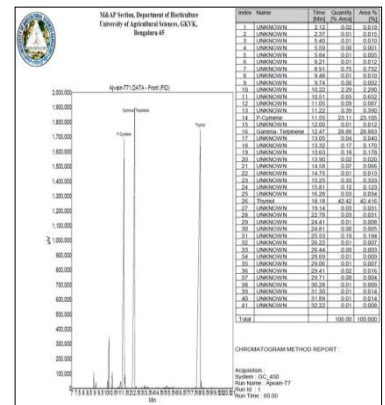


Fig 1d: ACC No. 7

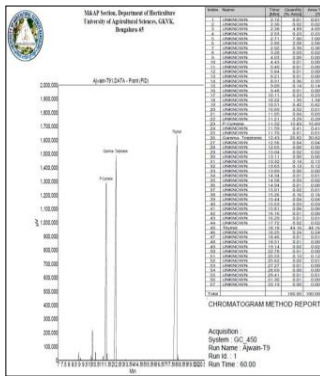


Fig 1e: ACC No. 9

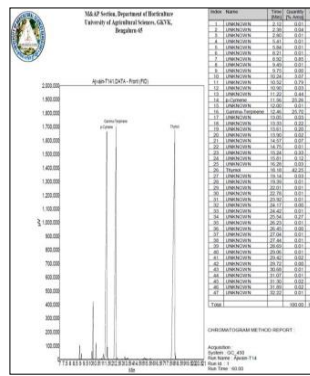


Fig 1f: ACC No. 14

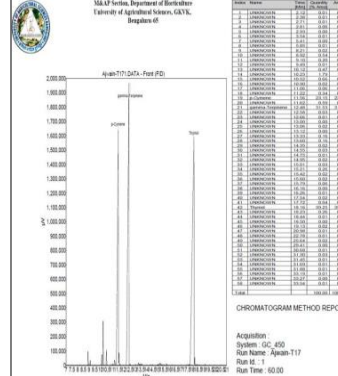


Fig 1g: ACC No. 17

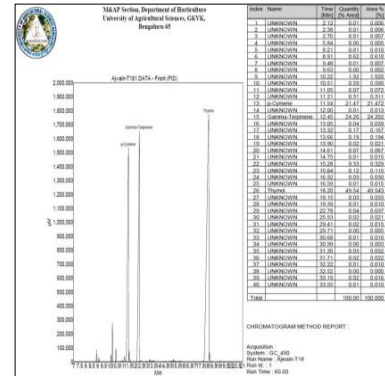


Fig 1h: ACC No. 18

Fig 1: P-Cymene, Y-terpinene and thymol content of different ajowan genotypes through gas chromatography

5. Acknowledgement

The authors gratefully acknowledge the financial support and guidelines from the Chairman Dr. L. Jeeva Jothi. Dr. M. Vasundhara, Professor (Hort.), UAS, GKVK, Bangalore thanks for GC analysis carried out the ajowan seeds.

6. Reference

1. Ajay Kumar CS, Raghav Chhokar SS, Khan IA. Composition and antimicrobial activity of ajowan (*Carum copticum* L.) seed essential oil from the different place in India. *Indian Perfumer*. 2008; 52:61-64.
2. Anna Riya. Evaluation of genotypes and effect of nutrient on clipping in coriander (*Coriandrum sativum* L.) Unpub. M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore, India, 2001.
3. Anoop Shetty A, Tanuja Buckseth, Naresh VS. Ajowan a under utilized seed spice. *Spice India*. 2012; 25(2):10-13.
4. ASTA. American Spice Trade Association. Official analytical methods. 5th Edn. New York. 1983; 38:9-10.
5. Ballba SI, Hilal SH, Huggag MY. The volatile oil from the herb and fruits of *C. copticum* at different stages of growth. *Planta Med*. 1973; 23:312-319.
6. Behravan J, Ramezani M, Hassanzadeh MK, Ebadi S. Evaluation of Antibacterial Activity of the Essential Oils of *Zataria Multiflora*, *Carum copticum* and *Thymus vulgaris* by a Thin Layer Chromatography-Bioautography Method. *JEOPB*. 2007; 10:259-264.
7. Bentley R, Trimen H. Medic

- Hook and *Semenovia tragoides* (BOISS) manden from Iran. J Essential Oil Res. 2002; 14:288-289.
19. Mirheidar H. Knowledge on Plants: Use of Plants in prevention and remedy of diseases. Daftar-e-Nashr Farhang Eslami, 1993, 2.
 20. Mohagheghzadeh A, Faridi P, Ghasemi Y. *Carum copticum* Benth. & Hook., Essential Oil Chemotypes. Food Chem. 2007; 100:1217-1219.
 21. Mohideen MK. Studies on variability, correlation and path analysis in (*Amaranthus gangeticus* L.). M.Sc., (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore, India, 1978.
 22. Pathak AK, Nainwal N, Goyal BM, Singh R, Mishra V, Nayak S *et al.* Pharmacological activity of *Trachyspermum ammi*: A Review. J of Pharmacol. Res. 2010; 3:895-899.
 23. Rajagopalan A, Azhakiamanavalan RS, Khader MDA. Evaluation of coriander cultivars for yield and quality. Indian Cocoa, Arecanut and Spices J. 1996; 20(1):13-14.
 24. Rajendran P. Variability studies in coriander (*Coriandrum sativum* L.). Unpub. M.Sc., (Ag.). Thesis, Tamil Nadu Agric. Univ., Coimbatore, India, 1978.
 25. Rina VK, Kumar A, Yadav A, Tandon S, Aggarwal KK. Composition of Indian ajowan (*Trachyspermum ammi* L.) seed oils. Indian Perfumer, 2004; 48(4):429-432.
 26. Saharkhiz MJ, Omidbaigi R, Sefidkon F. The effects of different harvest stages on the essential oil content and composition of Ajowan (*Trachyspermum ammi* L.) cultivated in Iran. J Essent. Oil Bearing Pl. 2005; 8(3):300-303.
 27. Sayre JK. Ancient herbs and modern herbs. Bottlebrush Press, San Carlos, California, USA, 2001, 14.
 28. Sharma RK, Bhatt DS. Performance of coriander varieties under irrigated condition. Indian Cocoa, Arecanut and Spices J. 1988; 11(3):96-98.
 29. Shazia Shabnum, Muzafar Wagay G. Essential Oil Composition of *Thymus vulgaris* L. and their Uses. J Res. Dev. 2011; 11:33-35.
 30. Singh VV, Sastry EVD. Mass, full and half sib- selection for genetic improvement in fennel (*Foeniculum vulgare* Mill.). J Spices and Arom. Crops. 2003; 12(2):179-182.
 31. Suthanthira Pandian IR, Shah HA, Muthuswami S. Genetic variability in coriander (*Coriandrum sativum* L.). Madras Agric. J. 1980; 67(7):450-452.
 32. Thangam C, Dhananjayan R. Anti-inflammatory potential of the seeds of *Carum copticum*. Indian J Pharmacol. 2003; 35:388-391.
 33. Umanath T. Evaluation and selection for grain and leafy types in coriander (*Coriandrum sativum* L.). Unpub. M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore, India, 1998.
 34. Zahin M, Ahmad I, Aqil F. Antioxidant and antimutagenic activity of *Carum copticum* fruit extracts. Toxicol. *In vitro*. 2010; 24:1243-1249.
 35. Zambonelli AD, Aulerio AZ, Severi A, Benvenuti S, Maggi L, Bianchi A. Chemical composition and fungicidal activity of commercial essential oils of *Thymus vulgaris* L. J Essent. Oil Res. 2004; 16:69-74.