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Introduction of mirtenal as an indicator component in essential oil of *Cuminum cyminum* Isfahan variety

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Key words: Cumin, Essential oil, Fertilizer , Cumin aldehyde , Myrtenal.

Abstract

Cumin *(Cuminum cyminum)* is one of the most important medicinal and aromatic plants which has high economic value in spite of its low yield. The seeds of *Cuminum cyminum* Isfahan variety were treated by six levels of chemical fertilizers and their essential oil was extracted by Clevenger. The essential oil yield was varied between 1.84% up to 5.06%. The essential oil was analyzed by GC/MS and their component was determined by use of Wiley electronic and NIST special libraries. There were seven major components with different percentage in different treatments. Major components were myrtenal (% 28-%43.5), β-pinene (%4.2-%20.65) , p-cymene (%4.4-%10.7), γ-terpinene (%13.65-%23.08), pulgen (trace- %7.46), Cumin aldehyde (16.59-27.8%) and p-menta,1-7,3al (%2.25-%9.6) .The high percentage of aldehyde in essential oil shows its suitable quality. In order to identify the essential oil of *Cumin* Isfahan variety from others, it could be referred to large amount of mirtenal as an indicator.

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Introduction

Cumin (*Cuminum cyminum*) belongining to Apiaceae family is an annual plant which growing to a height of 15- 30cm according to environmental condition . This plant has been known as one of the most important medicinal plants in Iran (Moraghebi and Aghelpasand, 2008). Adaptation to different environmental condition is one of its characters. In addition, culture and harvest time, low water and fertilization requirement and its high economic value makes it valuable in agriculture (Moraghebi and Etemadzadeh, 2006) . Cumin is produced in Iran, Lebanon, Cypress, Egypt, India, Syria , China, Indonesia , Mexico and Argentina (Karbacy. 2002) . Its cultivation has been restricted in Iran to margin of deserts in Khorasan, Isfahan and Kerman provinces (Kafi . 2002).

Agarwal identified 28 compound in essential oil which was extracted by Clevenger method. The major components were p-cymene (42.13%) and Cumin aldehyde (27.7%) (Hemati- Khakhaki and Senoei -Mohasese. 2002). In another work 30different components were identified in essential oil of cumin which was obtained by distillation method . The major components were β-pinene (18.7%), γ-terpinene (26%), Cumin aldehyde(12.2%), p-menta,1-7,3al (14.4%) and a combination of p-cymene , limonene and cineol (27.3%) (Sema. *et al* 2007). Comparison of essential oil components in Turkey extracted by distillation and steam method revealed 30differebnt components. The most important components were Cumin aldehyde (19.2%-27.8%), 1,3-p-menta,d-n-al (%4.3-12.3) , 1,4-p-menta,d-n-al (%24.5-44.9), γ-terpinene (%7-14) , p-cymene (%4.6-%12) and β-pinene (%3-%8.9) (Agarwal,*et al*.1979). In another study γ-terpinene (%29) , p-cymene (%25) β-pinene (%19.9) and Cumin aldehyde (1837%) were reported as the most important components of cumin essential oil (Baser *et al*., 1992). The most important combinations of Cumin Yazd variety are Propanol (%26.9), Benzene Methanol (%25.4), Phenyl Betanol (%16.49) and Gama-Terpene (%13.04) (Haghiroalsadat *et al*., 2011).

Cumin possesses numerous medicinal properties such as carminative, eupeptic and astringent. Their antimicrobial and antiseptic property has been known from ancient time (Borges and Pino, 1993). Study of antimicrobial activity of food additive such as cumin, cloves, red pepper and anise against *Staphylococcus aureus, Klebsiella pneumonia, Mycobacterium smegmatis, Micrococcus luteus, Enterococcus faecalis, Pseudomonas aeruginosa and Candida albicans showed* that cumin had the most activity (Rechinger. 1981). The most useful portion of cumin is its fruit which contains oil (7 %), resin (13%), essential oil (3.5-4%) and aloren . Essential oil which obtains from crushed fruits by distillation is colorless and sticky. It has a very strong odor with 0.91-0.93% density. The essential has 30% cuminol (4-isopropyl benzaldehyde) . The odor of essential oil and fruit originates from cuminol. Other components such as cymene, flanderin, cuminic alcohol are seen in cumin essential oil. The amount of other components is rare. Antimicrobial and antifungal activity of cumin depends on Cumin aldehyde (Judd and Campbell 1999).

The effect of fertilization on morphology of different varieties from Kerman, Khorasan and Isfahan indicated that Isfahan variety has special characters in compared to other varieties (Moraghebi and Aghelpasand 2008).

The morphologic differences could be a sign of a separation in the variety. Our purpose of this study is comparison between essential oil of *Cumin* Isfahan variety and other regions' *Cumin*, in order to find if there are physiologic differences (essential oil) beside the morphologic differences?

Methods and materials

The research farm of shahre-e-Rey Azad university which locates in 12 kilometer far from Tehran was chosen for spring plantation. The soil texture was

determined by baycas and the amount of N,P and K was measured by standards methods in Tehran soil and water research institute. The texture was clay without any nutrient limitation. Seeds were purchased from newly produced ones in Isfahan province. Seeds were planted in 5 march. Experiment was done using randomized complete block design in factorial with three replications. Harvesting was done when 70% of seeds were ripened. Nitrogen , potassium and phosphor fertilizers were used according to design. Urea $CO(NH2)_2$ containing 48% $NO_3.NH_4$, triple super phosphate containing 21% P_2O_5 and potassium chloride containing 52% K_2O were used as nitrogen potassium and phosphor fertilizer. Decomposed animal manure was used as organic fertilizer. Treatments includes a1 (N=20, P=16, K=20 kg/hectare) , a2 **(** N=40 P=32 K=40 kg/hectare) a3 **(** N=80 P=64 K=80 kg/hectare), b1 (15 tone animal manure in hectare) and b2 (25 tone animal manure in hectare). Controls did nor receive any fertilizers. Treatments were done during seed sowing. The half of urea was added during sowing and remainder was added after tillering(. Ahmadi, *et al* 2001).

Essential oil was extracted by Clevenger (water distillation). 30 gram of dried seeds in shadow were crushed and extracted for four hours in distillation apparatus. Extracted materials were collected in dark bottles. Essential oil was separated from water phase by decanter and stored in refrigerator after measuring weight. The amount of essential oil (ml) was estimated for 100 gram of dried seeds.

Essential oils analyzed by using Varian CP-300 gas chromatograph and GC-MS Varian q69drupole1200 equipped with a V7 column (30 m x 250 mm, film thickness 0.25 μm). The transfer line temperature was 280°C, carrier gas helium with a linear velocity of 1.5 ml /min, , ionization energy 70 ev, , mass range 33-400. Temperature was 50°-200 °C at a rate of 10°C /min. . The injector temperature was 280°C. The data were analyzes by excel and SAS software. Mean comparison was done by Duncan method.

Results

After harvesting, yield was determined in hectare. The average of essential oil was measured for three replications in treatments. Finally amount of essential oils were estimated in hectare according to yield and essential oil percent (table 1). Results showed that yield and essential oil characters was significantly (p< .01) affected by fertilization. In other hand, obtained essential was significantly (p< .01) affected by repetitions. This justifies the variance analysis (table 1) done by ANOVA method. Table 2 shows that the grouping of yield character in control differed from treatments. Treatment A2 (mean yield = 307.87) had the highest yield . The yield mean in control was the lowest (73.33) that indicates the improving effect of NPK fertilizer on cumin characters. B1 and B2 had the common grouping (c). In addition, essential production in the control (1.87 ml) was the lowest (group d). There were seven major components in essential oils which compromise 98% of it. Major components were β-pinene, p-cymene, γ-terpinene, pulgen (trace in three treatments), Cumin aldehyde, p-menta, 1-7,3al and myrtenal. (Figures 1, 2 and 3).Table 3 shows the essential oils composition. in different treatments. Results indicated that treatments had different effects on essential oil composition. β-pinene compromise 63% of essential oil in controls which was 2- 3 times less than its amount in a2 and a3 treatments.

Table 1. Analysis variance of treatments on studied characters in cumin.

Source of variation	ó freedom degree	Yield(Kg)	Essential oil (ml)
Treatment	5	19582.90**	3.516**
Block	2	779.03	0.744**
Error	10	324.77	0.088
Variation coefficient	-	9.38	9.141

: Means of squares are significant in 1% and 5% , respectively ** ,*

There was no difference in amount of p-cymene between control and treatments. γ-terpinene compromise 16.28 % of essential oil in control and treatment except to A2 and A3 treatments which its amount was more than its amount in control. The amount of pulgen was in fifth order but its amount in treatment was lower indicating that treatments caused removal or decreasing of its amount. Cumin aldehyde with 27.8% in control essential oil was the second major component. This component is seen in all treatments and had second or third important grade. A2 treatment had the lowest amount of Cumin aldehyde with 16.59 percentage. p-menta, 1-7,3al was in seventh grade . This component was seen in treatments with less than 10 percentages. Mirtenal was the major component in all treatments ranging from 28% in A3 to 43.5% in A1.

Table 2. Mean Comparison of yield and essential oil production by Duncan method.

Parameters	Yield(Kg)	Essential oil (ml)
Fertilization Treatments		
Control	73.33 d	1.8767 d
A1	226.73 b	2.7300 c
A2	307.87 a	3.8067 b
A3	230.07 b	2.9767 c
B1	157.07 c	3.0733 c
B2	156.57 c	5.0633 a

There was no significant difference(p<.05) among treatment with common letter.

Table 3. percentage of essential oil components in 6 fertilization treatments.

	Control	A1	A2	A3	B1	B2
β-pinene	6.3	4.2	20.65	13	8.9	9.8
p-cymene	7	4.4	7.98	6.3	10.7	6.08
γ-terpinene	16.28	16.44	23.08	22.5	26.6	13.95
pulgen	7.46	2.1	trace	0.8	trace	trace
Cumin aldehyde	27.8	23.9	16.59	19.5	23.5	26.86
p-menta, 1-7,3al	3.8	5.3	3.2	9.6	2.39	2.25
myrtenal	31	43.5	28.48	28	32.7	4.02

Discussion

Over time primary varieties might be divided to some varieties due to ecological specifications or mutation. Separation of varieties is with complex of genetic, morphologic, physiologic changes, etc. The results in this study indicate the physiologic separation of essential oil of *Cumin* Isfahan variety from other varieties.

Previous studies report the efficiency of seed essential oil between 2.5-3.6% (Mozaffarian. 2001). The efficiency of control was less than mentioned amount but treatments had the same efficiency as mentioned amount. The efficiency was 5.06% in B2 treatment that is considerable. Comparisons of our results on essential oil components percentage show some difference with others results (Mazandarani *et al.*2004).

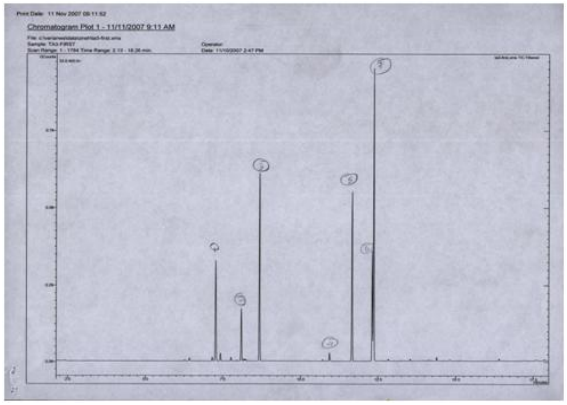

Fig. 1. Cumin essential oil chromatogram related to B2 treatment.

β-pinene, Cymene, γ-terpinene, pulgen, cumin aldehyde, p-menta, 1-7,3al percentage ranged from 4.2 to 20.65% , 4.4 to 10.7% , 13.95 to 26.6%, trace to 7.4% and 2.25 to 9.6% , respectively . The low amount p-Cymene indicates the good quality of essential oil. The presence of pulgen in cumin essential oil has been not reported by others. P-menta, 1-7,3al makes an undesirable odor of essential oil and its low amount improves the quality of essential oil.

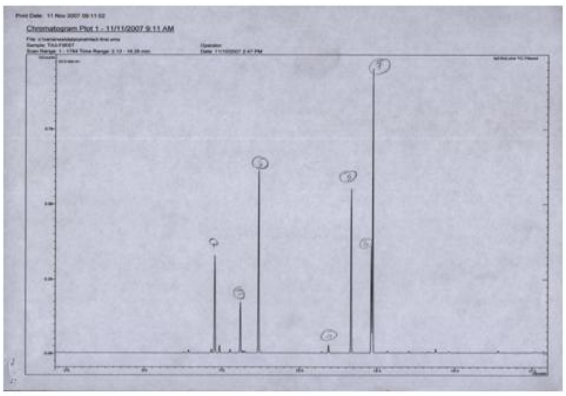

Fig. 2. Cumin essential oil chromatogram related to ÕA3 treatment.

While others have not reported the myrtenal as a component of cumin essential oil, we report its percentage from 28 to 43%.

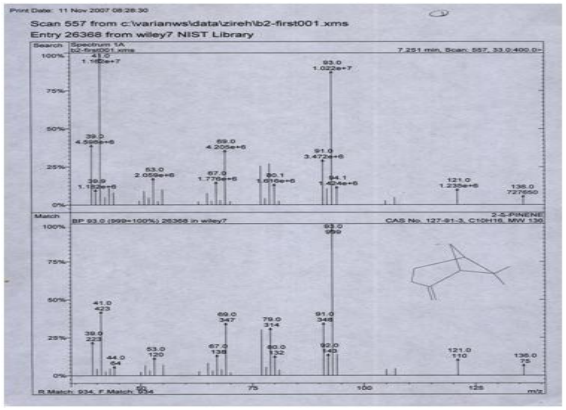

Fig. 3. Mass spectrum of β-pinene.

It seems that Isfahan variety is botanically different from Kerman, Khorasan_Gorgan and Yazd varieties due to the presence of Pulgen and myrtenal in Isfahan variety essential oil that has been reported in some varieties of cumin and their absence in Kerman, Yazd and Khorasan_Gorgan varieties (Ahmadi *et al.*, 2001; Mazandarani *et al.*, 2004; Haghiroalsadat *et al.*, 2011). This botanically difference has been reported by Moraghebi and Aghelpasand (2008). This botanically difference has been reported by Mozafarin (2004) who believes that only *Cuminum cyminum* exists in Iran while Rechinger (1981) reported a subspecies under title of *Cuminum setifolium*. RashedMohsal (2002) reported two species including *Cuminum cyminum* and *Cuminum setifolium*.

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