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ORIGINAL RESEARCH ARTICLE

Harvest and post-harvest studies on *Mentha arvensis* var. CIM-Kranti for quality essential oil production in winter and summer cropping seasons

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ABSTRACT

Menthol-mint (M. arvensis) is cultivated worldwide for its essential oil, menthol and dementholized oil, widely used in flavour, fragrance and pharmaceutical industries. Numerous varieties have been developed for the large scale cultivation of M. arvensis in India to meet out its quality essential oil production. The present experiment was designed to study the influence of cropping seasons, harvesting time, drying conditions and drying periods on the yield and quality of essential oil of the prevalent variety 'CIM-Kranti' of M. arvensis grown at the foot hills agro-climatic conditions of northern India. Essential oil content varies from 0.9-1.1% and 0.6-0.7% in summer and winter crops, respectively. Altogether, a total of 21 chemical constituents, comprising 96.2-99.5% of the essential oil, were identified using Gas Chromatography–Flame Ionization Detector (GC-FID) and Gas Chromatography–Mass Spectrometry (GC-MS) analysis. Menthol (68.7-83.4%), menthyl acetate (2.5-5.3%), menthone (3.1-8.6%), and isomenthone (3.4-5.9%) were identified as the major constituents in both cropping seasons. Significant quantitative variation in yield and content of menthol was noticed as a function of harvesting periods and drying conditions in summer and winter crops of M. arvensis var. CIM-Kranti.

INTRODUCTION

Members of the genus *Mentha* L., commonly known as mints, are annual or perennial, aromatic herbs belong to family *Lamiaceae*. The genus *Mentha* constitutes ca. 62 taxa belonging to 18 species and 11 hybrids that are distributed worldwide (Bajeli et al., 2016). The members of this genus are mainly cultivated in the temperate, Mediterranean, and subtropical climates of America, Europe, China, Brazil, India etc. for their culinary and medicinal applications (Lawrence, 2007; Padalia et al., 2013) . The most popular and commercially cultivated mints are menthol-mint (*Mentha arvensis* L.), peppermint (*Mentha* ×

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piperita L.), spearmint (*Mentha spicata* L.), bergamot-mint (*Mentha citrata* Ehrh.), scotch-spearmint (*Mentha cardiaca* Bak.), and pennyroyal mint (*Mentha pulegium* L.). Menthol-mint (*M. arvensis*), one of the most important member of this genus, is cultivated in a wide range of soil types and environmental conditions of tropical and subtropical climates across the globe. It is cultivated mainly for its essential oil, menthol, dementholized oil (DMO), mint terpenes and terpeneless DMO etc. which are used extensively in food-flavour, fragrance, cosmetics and pharmaceutical industries (Bajeli et al., 2016; Lal, 2013; Rao and Syamasundar, 2013; Singh et al., 2005). In India, menthol-mint is cultivated in more than 200,000 hectares area in Uttar Pradesh, Bihar, Haryana, Punjab, West Bengal, Jammu and Kashmir, Himachal Pradesh and Uttarakhand (Rao and Syamasundar, 2013; Shukla et al., 2011). India is the leading producer, consumer and exporter of menthol-mint oil in world market at present with more than 80% of global share due to suitable agroclimatic conditions and development of prevalent high essential oil yielding varieties. The existing varieties of menthol-mint developed in India for large scale cultivation are CIM-Kranti, CIM-Saryu, Kosi, Kushal, Himalaya, Kalka, Damroo, Gomti, Saksham, Sambhav, and MAS-1. In India, usually menthol-mint is cultivated as a summer crop, during January-February to May-June, using suckers (stolons) and/or during March-April to May-June by transplanting with plantlets raised in nursery (Chauhan et al., 2009; Padalia et al., 2015; Rahman et al., 2014; Singh and Khanuja, 2007). The menthol-mint variety 'CIM-Kranti' is a cold and frost tolerant variety, developed for both winter and summer cropping seasons. This variety produces 10-15% higher essential oils compared to the other prevalent cultivars in summer season. Moreover, it has the potential to produce ~ 100 kg ha^{-1} oil having 80% menthol in the winter season compared to other varieties which suffer senescence by the cold and frost conditions in the winter season (Bahl et al., 2013). Hence, 'CIM-Kranti' variety has become popular among mint growing farmers in India as a prominent source of menthol-mint oil in both winter and summer seasons. Considering its potential for both summer and winter cropping seasons, this study was conducted with the following objectives: (i) to evaluate yield and essential oil quality of summer and winter crop of *M. arvensis* var. CIM-Kranti harvested at different time intervals (8 am, 11 am, 2 pm and 5 pm) in a day; and (ii) to evaluate the influence of drying methods (in shade and field conditions) and drying periods (24 h to 72 h) on essential oil quality of under foothills agro-climatic conditions of northern India.

MATERIALS AND METHODS

Plant material and essential oil extraction

The field experiment was conducted at the experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, Uttarakhand, India located at coordinates 29° N, 79.38° E, at an altitude of 243.84 m in the foothills of north India. To evaluate the essential oil yield and quality of *M. arvensis* var. CIM-Kranti harvested at different time intervals (8 am, 11 am, 2 pm and 5 pm) in a day, matured crops of two cropping seasons (summer season and winter seasons was harvested). The climate of this site is sub-tropical and humid. For the summer crop, the suckers of *M. arvensis* var. CIM-Kranti were planted in the first week of February and the crop was raised following normal agricultural inputs. However, for winter crop, the shoots were planted in the first week of August following normal agricultural inputs. Fresh samples were harvested after 120 days of planting at different time of the day (8 am, 11 am, 2 pm and 5pm) for distillation in the first week of June for summer crop, and the first week of January for winter crop. To evaluate the effect of drying methods and periods on compositional variability in essential oils of *M. arvensis* var. CIM-Kranti, the samples were harvested at 11:00 am in summer and winter crops were dried in shade and field conditions from 24h to 72h prior to distillation. The harvested samples of *M. arvensis* were hydrodistilled in Clevenger type apparatus for 3 h, in triplicate, for extraction of essential oil. The oils were dried over anhydrous sodium sulphate and stored in refrigerator prior to further analysis.

Analysis of the essential oil

The essential oil samples were analysed by GC-FID and GC-MS techniques. GC-FID analysis of the essential oils were carried out on Nucon gas chromatograph (Model 5765) equipped with DB-5 capillary column (30 m × 0.25 mm, with 0.25 µm film thickness) and on Thermo Fisher Trace GC-1300, equipped with TG-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness), with flame ionization detector (FID). The oven column temperature ranged from 60 to 230°C, programmed at 3°C min^{-1}, using H_2 as carrier gas at 1.0 mL min^{-1} for Nucon gas chromatograph, and 70–230°C, programmed at 4°C min^{-1}, with N_2 as the carrier gas at constant flow rate of 1.0 mL min^{-1} for Thermo Fisher Trace GC. The injector and detector (FID) temperatures were maintained at 220°C and 230°C, respectively. Injection volumes of the oils were 0.02 µL neat with a split ratio of 1:40. The relative content of constituent was calculated based on the peak area (FID response) without using a correction factor. GC-MS of the essential oil was performed on Clarus 680 GC coupled with Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 fused silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm). The oven column temperature program was from 60 to 240°C at 3°C min^{-1} with initial and final hold time 2 min. Helium was used as carrier gas at 1.0 mL min^{-1}. The injection volume was 0.02 µL neat with a split ratio of 1:30. The injector and ion source temperatures were maintained constant at 250°C. The ionization energy was 70 eV (EI), with a mass scan range of 40-400 amu.

Identification of essential oil constituents

The essential oil constituents were identified by comparing retention indices (RI, determined with homologous series of *n*-alkanes, C_8-C_{24}, Supleco Analytical, Bellefonte PA, U.S.A.), mass spectra library search (NIST and Wiley) and by comparing their mass spectra (MS) data with literature data (Adams, 2007).

RESULTS AND DISCUSSION

The essential oil yield and composition of *M. arvensis* var. CIM-Kranti harvested at different time intervals (8 am, 11 am, 2 pm and 5 pm) in a day and wilted in shade and field conditions upto 72 h in both summer and winter cropping seasons are summarized in Tables 1 and 2. The essential oil yield (%), determined by hydrodistillation method on fresh weight basis were found to vary from 0.9-1.1% and 0.6-0.7% in *M. arvensis* harvested at different time period in summer and winter cropping seasons, respectively. The essential oil yield of *M. arvensis* in the summer season was found to show slight changes as per the harvesting time in the order: 1.1% (2.00 pm) > 1.0% (11.00 and 8.00 am) > 0.9% (5.00 pm). However, in winter crop the essential oil yield was as per the order: 0.7% (11.00 am and 2.00 pm) > 0.6% (8.0 am and 5.00 pm). Although the variation seems to be numerically minor (0.1-0.2%), this has a huge consequence for farmers and industry for total essential oil production, when the menthol mint is cultivated in larger area (mainly in hectares). The changes in essential oil yield of *M. arvensis* var. CIM-Kranti during the day due to diurnal variations of photoperiods, relative humidity, sun light intensity, and temperature are in agreement with previous reports on various aromatic crops *viz*. *R. damascena, C. citratus, C. winterianus, Ocimum basilicum, O. gratissimum, O. kilimandscahricum, O. selloi, L. stoechas, C. sativum, T. vulgaris, L. alba*, and *R. officinalis*. (Blank et al., 2007; Chang et al., 2009; Kaya et al., 2013; Kaya et al., 2012; Kumar et al., 2013; Ramezani et al., 2009; Yilmaz et al., 2011). The essential oil quality of *M. arvensis* var. CIM-Kranti harvested at different time intervals during the day and dried as per experimental protocol were analyzed and compared using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of twenty-one constituents, representing 96.2-99.5% of the total oil composition were identified by comparing their retention indices and mass spectral data. Qualitative variations of these 21 constituents in the analysed essential oils of *M. arvensis* harvested at different periods and different drying conditions are summarized in Tables 1 and 2. Monoterpenoids (94.9-98.1%) represented by menthol (68.7-83.3%), menthone (3.3-8.6%), isomenthone (3.5-5.9%) and menthyl acetate (2.5-

5.3%) are the major constituents of the essential oils of *M. arvensis* harvested at different time intervals in a day in summer and winter cropping seasons. The content of menthol varies from 80.8-83.3% and 68.7-74.0% depending on the harvesting period of the *M. arvensis* crop in summer and winter seasons, respectively. The maximum menthol content (83.3%) was noticed when the crop was harvested at 11 am, followed by 8 am (82.4%), 5 pm (81.4%) with a minimum at 2 pm (80.8%) during the summer season. Similar trend was also noticed during the winter season, with maximal menthol content (74.0%) at 11 am followed by 8 am (72.6%), 5 pm (71.4%) with minimum at 2 pm (68.7%). Other constituents also showed quantitative variations as per the harvesting period during the day (Table 1, Figs. 1, 2). Moreover, to determine the influence of the drying methods and wilting periods on the quantity and quality of the essential oil of *M. arvensis* var. CIM-Kranti in both summer and winter cropping seasons, the harvested crop after 120 days of planting was left in the shade and in the open field condition upto 72 h after harvesting (Table 2, Figs. 3, 4). The essential oil quality was found to be better in shade-dried conditions with high content of menthol (83.1-83.4%), compared to the field drying condition with lesser menthol content (81.2-81.8%) in summer cropping season. Similar trend in essential oil quality was noticed in winter cropping season, when the harvested crop was left in shade and field conditions for wilting for different periods. However, the content of menthol in shade and field dried condition showed quantitative variations depending on the wilting period from 24-72 h. During shade drying conditions in summer season, the menthol content follow the order as: 24h (83.4%) > 48h (83.3%) > 72h (83.1%); whereas in field drying conditions the menthol content follows

Table 1: Chemical composition of the essential oil of *M. arvensis* var. CIM-Kranti harvested at different time during the day in summer and winter crops

Compounds[a]	RI[b]	RI[c]	Content (%)							
			Summer season				Winter season			
			8:00 am	11:00 am	2:00 pm	5:00 pm	8:00 am	11:00 am	2:00 pm	5:00 pm
α -Pinene	932	932	t	t	t	t	0.7	0.6	0.7	0.6
Camphene	946	946	0.3	0.2	0.3	0.1	t	t	t	t
Sabinene	971	969	0.3	0.4	0.4	0.4	0.3	0.3	0.2	0.2
β –Pinene	974	974	0.2	0.2	0.2	0.2	0.7	0.6	0.7	0.6
Myrcene	995	988	t	t	t	t	0.6	0.5	0.6	0.5
Limonene	1020	1024	1.5	1.8	1.9	1.4	3.1	3.3	3.9	3.5
Linalool	1093	1095	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Camphor	1137	1141	0.3	0.3	0.3	0.3	0.8	0.7	0.8	0.8
Menthone	1142	1148	**3.3**	**3.6**	**4.4**	**3.5**	**8.3**	**7.9**	**8.6**	**7.9**
Isomenthone	1157	1158	**3.6**	**3.5**	**4.0**	**3.9**	**5.7**	**5.8**	**5.9**	**5.4**
Menthol	1162	1167	**82.4**	**83.3**	**80.8**	**81.4**	**72.6**	**74.0**	**68.7**	**71.4**
α- Terpineol	1184	1186	0.1	-	0.1	0.1	0.2	0.2	-	0.2
Pulegone	1232	1233	t	t	0.2	0.1	t	0.1	t	t
Piperitone	1248	1249	0.5	0.5	0.3	0.3	0.5	0.4	0.5	0.4
neo-menthyl acetate	1270	1271	0.1	t	0.1	t	t	t	0.1	0.1
Menthyl acetate	1293	1294	**3.4**	**4.2**	**3.0**	**3.1**	**3.4**	**2.5**	**5.3**	**4.7**
β-Caryophyllene	1414	1417	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.1
Germacrene D	1584	1484	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.3
Elemol	1554	1548	0.7	0.7	0.7	0.7	0.1	0.1	0.1	0.1
Spathulenol	1574	1577	0.1	0.2	0.2	0.2	t	t	0.1	t
Caryophyllene oxide	1586	1582	t	t	t	t	0.2	0.1	0.1	t
Class compositions										
Monoterpene hydrocarbons			2.3	2.6	2.8	2.1	5.4	5.3	6.1	5.4
Oxygenated monoterpenoids			93.8	95.5	93.3	92.8	91.6	91.7	90	91
Sesquiterpene hydrocarbons			0.5	0.5	0.5	0.4	0.5	0.6	0.6	0.4
Oxygenated sesquiterpenoids			0.8	0.9	0.9	0.9	0.3	0.2	0.3	0.1
Total identified			**97.4**	**99.5**	**97.5**	**96.2**	**97.8**	**97.8**	**97.0**	**96.9**
Essential oil content (%)			**1.0±0.06**	**1.0±0.14**	**1.1±0.03**	**0.9±0.08**	**0.6±0.06**	**0.7±0.0**	**0.7±0.08**	**0.6±0.15**

[a]Mode of identification: Retention Index (RI), MS (GC-MS); [b]Experimental retention index determined on TG-5 gas chromatography capillary column (30 m × 0.25 mm) using n-alkanes; [c]Retention index from the literature (Padalia et al., 2013); t = trace (<0.05 %)

Table 2: Effect of drying conditions on essential oil quality of *M. arvensis* var. CIM-Kranti harvested during summer and winter season

Compounds[a]	RI[b]	RI[c]	Content (%)											
			Summer season						Winter season					
			Shade dried			Field dried			Shade dried			Field dried		
			24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
α -Pinene	932	932	-	-	-	-	-	-	0.7	0.7	0.7	0.7	0.6	0.7
Camphene	946	946	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.6	t	t	t
Sabinene	971	969	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.2	0.2	0.3
β –Pinene	974	974	-	0.2	0.2	0.2	0.1	0.2	0.7	0.6	0.7	0.6	0.6	0.7
Myrcene	995	988	t	t	t	t	t	t	0.3	0.6	0.6	0.5	0.5	0.6
Limonene	1020	1024	1.5	1.4	1.6	1.9	1.8	1.5	2.6	3.1	3.1	3.3	2.6	3.2
Linalool	1093	1095	t	t	0.1	0.1	0.1	0.1	t	0.1	t	t	t	t
Camphor	1137	1141	0.3	0.3	0.3	0.2	0.3	0.2	0.8	0.7	0.7	0.8	0.7	0.6
Menthone	1142	1148	**3.1**	**3.2**	**3.5**	**4.3**	**4.0**	**3.3**	**7.2**	**7.9**	**8.3**	**8.1**	**6.0**	**7.6**
Isomenthone	1157	1158	**3.6**	**3.4**	**3.5**	**3.5**	**3.7**	**3.4**	**5.4**	**5.6**	**5.4**	**5.4**	**5.2**	**5.2**
Menthol	1162	1167	**83.4**	**83.3**	**83.1**	**81.2**	**81.5**	**81.8**	**72.8**	**73.2**	**73.1**	**72.1**	**73.2**	**71.7**
α- Terpineol	1184	1186	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
Pulegone	1232	1233	0.1	t	0.1	t	0.1	t	0.1	0.1	0.2	t	t	0.1
Piperitone	1248	1249	0.3	0.4	0.4	0.3	0.4	0.4	0.5	0.5	0.5	0.4	0.5	0.3
neo-menthyl acetate	1270	1271	t	t	t	t	t	t	t	t	t	t	t	t
Menthyl acetate	1293	1294	**3.0**	**3.0**	**3.0**	**4.0**	**4.0**	**5.1**	**4.4**	**4.2**	**3.7**	**5.0**	**4.1**	**5.0**
β-Caryophyllene	1414	1417	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2
Germacrene D	1584	1484	0.3	0.2	0.2	0.2	0.2	0.3	0.4	0.1	0.4	0.3	0.2	0.2
Elemol	1554	1548	0.9	0.7	0.8	0.7	0.8	0.6	0.7	0.5	0.1	0.4	0.4	0.4
Spathulenol	1574	1577	0.2	t	t	0.2	0.2	0.2	t	0.1	t	0.1	0.1	0.1
Caryophyllene oxide	1586	1582	t	-	t	t	-	t	t	t	-	t	t	-
Class compositions														
Monoterpene hydrocarbons			2.1	2.1	2.4	2.7	2.5	2.3	4.8	5.5	6	5.3	4.5	5.5
Oxygenated monoterpenoids			93.9	93.7	94.2	93.7	94.2	94.4	91.4	92.5	92.1	92	89.9	90.7
Sesquiterpene hydrocarbons			0.6	0.4	0.4	0.5	0.5	0.6	0.7	0.4	0.6	0.5	0.5	0.4
Oxygenated sesquiterpenoids			1.1	0.7	0.8	0.9	1.0	0.8	0.7	0.6	0.1	0.5	0.5	0.5
Total identified			**97.7**	**96.9**	**97.8**	**97.8**	**98.2**	**98.1**	**97.6**	**99.0**	**98.8**	**98.3**	**95.4**	**97.1**
Essential oil content (%)			**1.0± 0.03**	**1.0± 0.0**	**1.0± 0.03**	**0.9± 0.04**	**0.9± 0.03**	**1.0± 0.08**	**0.6± 0.03**	**0.7± 0.08**	**0.7± 0.03**	**0.7± 0.22**	**0.7± 0.11**	**0.7± 0.08**

[a]Mode of identification: Retention Index (RI), MS (GC-MS); [b]Experimental retention index determined on TG-5 gas chromatography capillary column (30 m × 0.25 mm) using n-alkanes; [c]Retention index from the literature (Padalia et al., 2013); t = trace (<0.05 %)

the reverse trend: 24h (81.2%) < 48h (81.5%) < 72h (81.8%). However, in shade drying conditions in winter season, the menthol content was maximal at 48 h (73.2%) > 72h (73.1%) > 24h (72.8%); whereas, in field drying conditions the content of menthol follow the order: 48h (73.2%) > 24h (72.1%) > 72h (71.7%). As earlier reported for various aromatic crops, the changes in essential oil yield and quality of essential oils during different time periods of the day are due to varying degree of daily fluctuations in interdependent factors such as temperature, humidity, intensity and photoperiods etc.(Chang et al., 2009; Kumar et al., 2013; Padalia et al., 2017; Sousa et al., 2010).

In conclusion, the summer cropping season is better than the winter season for *M. arvensis* var. CIM-Kranti, in terms of essential oil yield and menthol content. While, wilting of the harvested crop in the field upto 72 h had no adverse effect on the essential oil yield and quality, 72 h of wilting prior to distillation can reduce the fuel consumption and number of batches for distillation that could be an economic significance for farmers.

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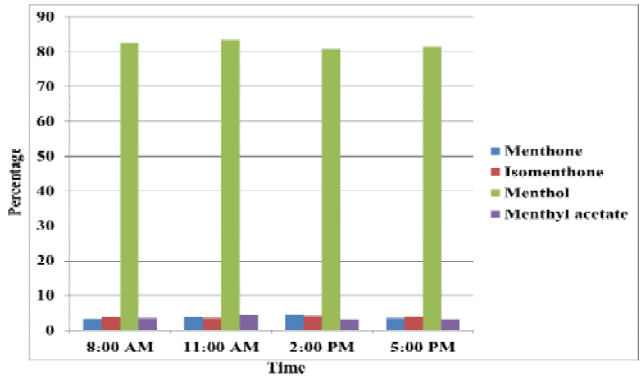

Figure 1: Chemical composition of the essential oil of *M. arvensis* var. CIM-Kranti harvested at different time in summer cropping season

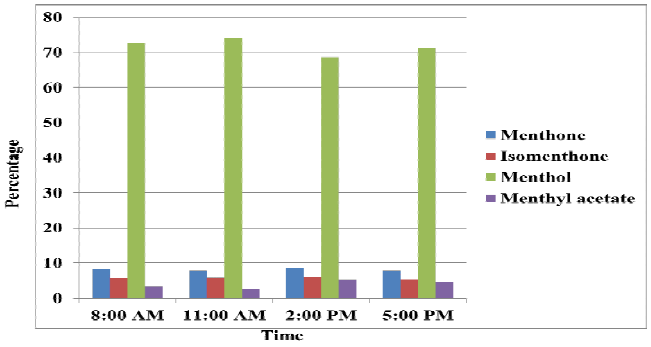

Figure 2: Chemical composition of the essential oil during *M. arvensis* var. CIM-Kranti harvested at different time during winter cropping season

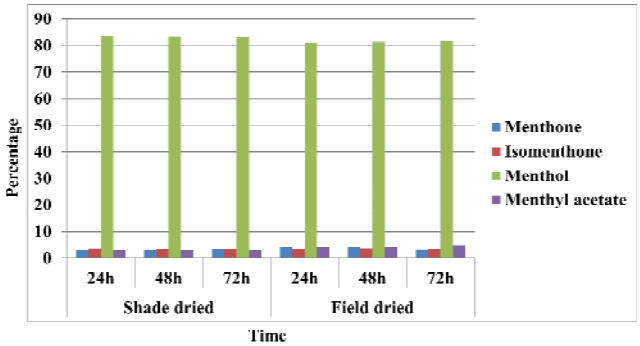

Figure 3: Effect of drying conditions and periods on essential oil quality of *M. arvensis* var. CIM-Kranti harvested during summer cropping season

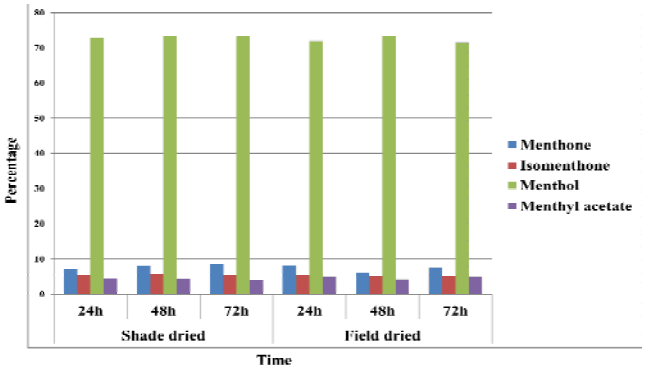

Figure 4: Effect of drying conditions and periods on essential oil quality of *M. arvensis* var. CIM-Kranti harvested during winter cropping season

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