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FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS OF OIL OF *MENTHAARVENSIS* GROWN AT SITES VARYING WITH VEHICULAR TRAFFIC LOADS IN LUCKNOW CITY, INDIA

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Abstract

The demand of the essential oil of mint species; widely used in food, pharmaceutical and cosmetic industries, is growing throughout the world. Owing its significance, it was felt important to know the changes in chemical characteristics of the oil, if any, for economic value when the crop of *Mentha* is grown near highways, railway tracks or areas having heavy traffic loads. To assess the effect of vehicular emissions on menthol (mint oil), transfer experiment study was conducted. Firstly, within the municipal premises of Lucknow city, five sites (Road stretches) were identified based on survey of Lucknow city and the available data on air pollution loads. Sites were selected which were differing from each other significantly in terms of the number of vehicles (source of pollution) plying on them but were quite similar to each other in other eco-physiological factors. On the select sites equal number of potted *Menthaarvensis* plants of the same age, height and vigour of saccham variety obtained from CSIR-CIMAP was kept to get exposed to auto-exhaust pollutants for one year. Irrigation regime at all sites was kept uniform to avoid the influence of any other variable other than vehicular emissions. An analysis of hydro-distillated essential oil of *Menthaarvensis* variety Sascham under FTIR (Fourier Transform Infrared Spectroscopy) revealed some qualitative changes in the organic-compounds of the oil from plants grown at sites of Lucknow city loaded with high vehicular load (auto-exhaust pollutants) over plants kept under relatively pollution free site. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. The quantity of the peppermint oil extracted from plants of site having highest traffic loads, in turn maximum ambient pollutants (NO^2, SO^2, O^3, SPM & RSPM) was also found less as compared to plants grown in less polluted site.

Keywords: FTIR, *Menthaarvensis*, Superannuated, F-45 KAAVISH, Sarvodaya Nagar, Lucknow

Introduction

Plants have been a potential source of medicine; though in a crude form, have been used from time immemorial to heal various ailments. A variety of bioactive compounds that are present in different parts of a plant has spurred a renewed interest in developing an alternate therapy. The traditional herbal medical system has been practiced globally from ancient times (Pramila et.al 2012). The mint species have a great importance, both medicinal and commercial. Indeed, leaves, flowers and stems of *Mentha spp.* are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavour. In addition, *Mentha spp.* has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its antinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities (Hadjlaoui, et.al. 2009). It is mainly grown for its leaves and essential oil .its leaves are used for herbal preparations (Beemnet et.al. 2010), and the essential oil extracted is used in many food industry (Verma et.al. 2010), because of its main component menthol and menthonone (Gul, 1994). Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition.

It has been recognized for a long time that IR spectroscopy is a powerful tool by which many individual functional groups can be identified and quantitatively analyzed due to their absorption at specific wavelengths in the infrared portion of the spectrum. The range of infrared spectra in IR spectroscopy spreads from about 4000 to 500 cm^{-1}. The complexity of infrared spectra in the 1450 to 600 cm^{-1} region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the fingerprint region. Absorption bands in the 4000 to 1450 cm^{-1} region are usually due to stretching vibrations of diatomic units and this is sometimes called the group frequency region, divided to 3 regions according to single-bond, triple-bond and double-bond molecules (Figure 1).

The aim of using FTIR analysis is to determine the existence of functional groups that exists in the oil and also the effect of pollution on the constituents of essential oils of *Menthaarvensis* cultivated in the field near highways or railways tracks or the fields near by heavy traffic load.

Materials and Methods

1.1. Study Area

The Lucknow city in the central plain of the Indian subcontinent is situated between 26°52_N latitude and 80°56_E longitude, 120 m above the sea level.The temperature of the city ranges from a minimum of 5°C in winter to a maximum of 47°C in summer with the mean average relative humidity 60% and the average rainfall 1,006.8 mm.

To conduct our study there will be five sites were selected on the basis of different vehicular load. They are:

1. University Campus (less polluted site)
2. Hazratganj intersection (busiest road)
3. Charbagh Railway Station

4. Transport Nagar Road, near Amausi industrial area, Lucknow-Kanpur Highway
5. GIMS, Lucknow-Raebareilly Highway

1.2. Design of Transfer Experiment

Roots of certified variety *i.e*. Sascham of *Menthaarvensis* were obtained from CIMAP, Lucknow and sown (2 roots per pot) in earthen pots of diameter 24 cm filled with garden soil and organic manure (3:1 soil: manure). After 15-20 days on the appearance of new leaves (a sign of plants establishment), pots were thinned by removing one and allowing one plant per pot and were transferred to select sites for the transfer experimental study. At each site fifteen pots were kept.

1.3. Air Quality Monitoring

Air quality monitoring was carried out twice a week during peak traffic hours using a portable high volume sampler for SPM and an attachment device (impingers) with sampler was used for monitoring gaseous pollutants. Using different absorbants: pararosaniline for SO_2, Sodium hydroxide arsenite for NO_2 and potassium iodide forO_3, the three major auto exhaust pollutants were measured.

Table 1- Air Quality Monotoring (Parametres and Methodology).

S.No.	Parameters	Time Weighted average	Methods of Measurement
1	Particulate Matter	8 hours twice a week	Gravimetric
2	Sulphur dioxide (SO_2)	8 hours twice a week	West Gaeke, 1956
3	Nitrogen dioxide(NO_2)	8 hours twice a week	Jacob &Hochhesier, 1958
4	Ozone (O_3)	4 hours twice a week	Byers and Saltzman, 1958

1.4. Extraction of the essential oil

100 g of the aerial parts of the Menthol mint were subjected to hydro-distillation for 2- 3 hour with 500 ml distilled water using a Clevenger-type apparatus. The oil obtained was collected and over stored in screw capped glass vials in a refrigerator. Yield based on fresh weight of the samples was calculated.

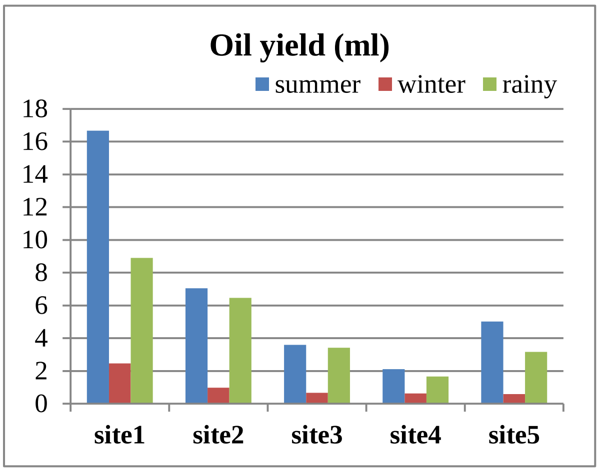
2. Results And Discussion

Ambient air quality monitoring showed significant spatial variations of the pollutants in suburban area of the city (**Table 1**). Charbagh (Site 3) and Transport Nagar (Site 5) was highly charged with pollutants coming out of vehicles emission, followed by SGPGI (Site 5) and Hazratganj intersection (Site 2), while BBA university campus (Site 1) showed very low levels of pollution. It was observed that the concentrations of all the pollutants were maximum during day time between 10 to 11 a.m. and 4 to 5 p.m. due to various urban activities (peak traffic hours).

Table 2. Concentration (µg m^3) of pollutants at selected sites (during April 2010 to February 2011)

Sites	Concentration (µg m^3) of pollutants at selected sites				
	SO_2	NO_2	O_3	SPM	RSPM
Site 1	08.07± 0.29	10.57±5.01	27.33± 5.25	87.23±6.47	59.07±6.53

Site 2	23.93±4.17	33.63±6.11	143.43±17.71	366.83±65.16	182.93±22.78
Site 3	47.37±8.91	49.13±6.79	154.63±10.04	447.03±29.97	239.87±11.30
Site 4	52.07± 4.24	48.57±9.12	131.63±15.30	465.57±29.12	268.47±16.72
Site 5	36.23±4.88	38.97±4.63	125.50 ±7.89	354.93±60.87	174.03±10.08



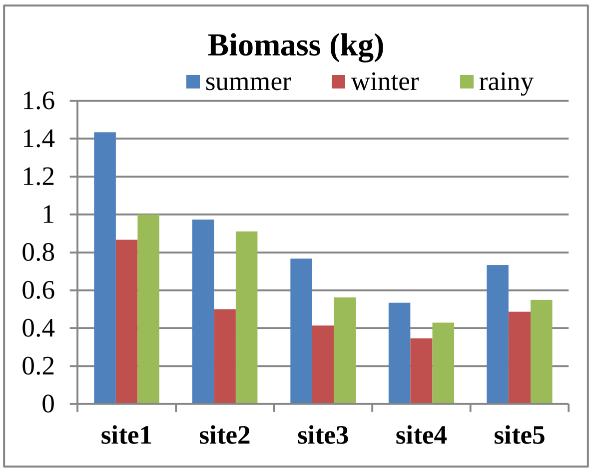
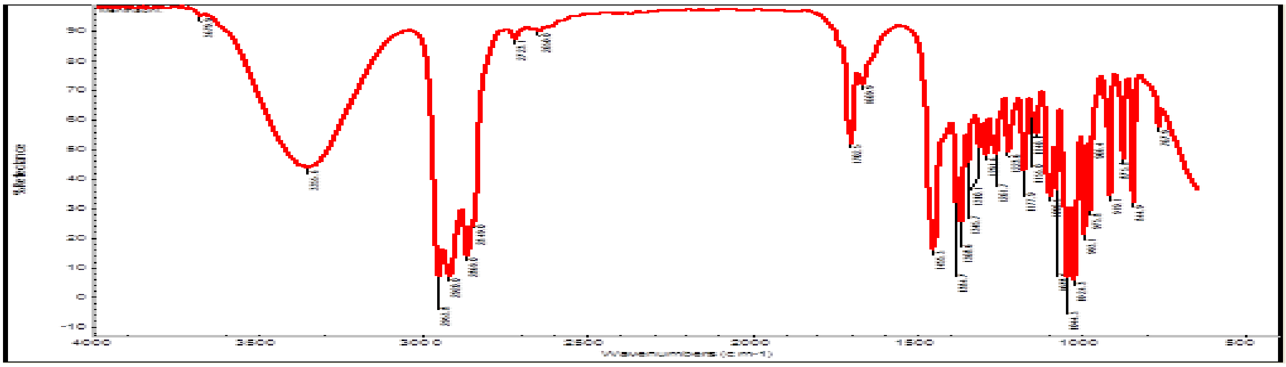


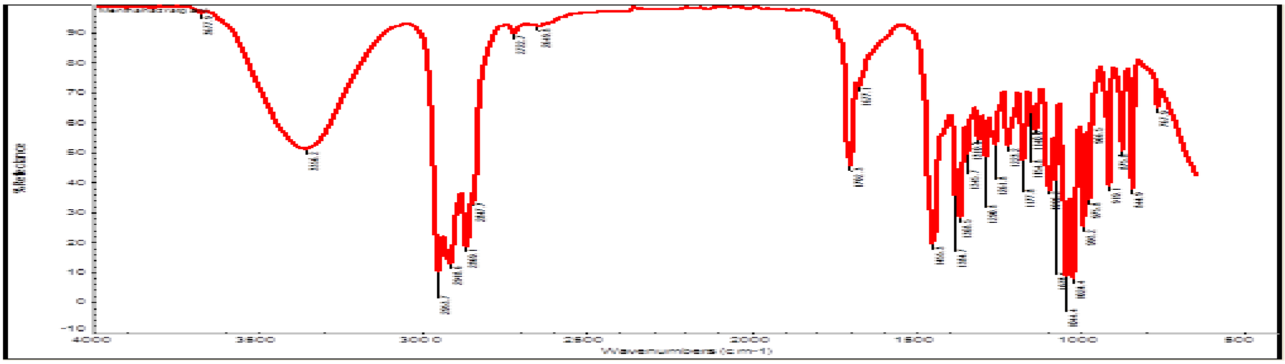
Fig 1. Oil yield (ml) and Biomass (kg) of *Menthaarvensis* at selected sites
[Values are mean ±SD (n=3)]

The essential oil contents of *M. arvensis*samples varied between 2.2 ml/kg to 16 ml/kg in summer, 2.41 to 0.58 ml/kg in winter and 8.91 to 1.66 ml/kg in rainy season. Maximum yield was recorded at site 1 and the minimum was recorded at site 4 in all seasons except in winter season were minimum yield were found at site 5(figure 1). Biomass (kg) of plant also shows the same pattern of reduction, *i.e*. Maximum at site 1 and minimum at site 5 (**Fig 1**).

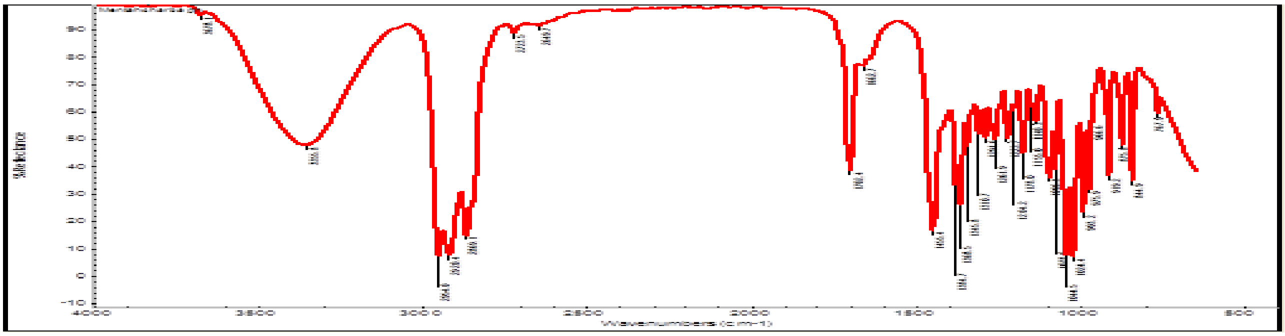
Oil concentration is influenced by environmental factors, methods of cultivation, seasonal variations and harvesting time (Qiu*et al*., 2005; Maric*et al*., 2006; Bernotienė*et al*., 2007). Also Qiu*et al.* (2005) reported qualitative and quantitative differences in the oil of *S. officinalis*collected in Shanghai, China at various seasonal periods. The increasing effect on essential oil contents during the vegetative stage of plant cycle could also be influenced by light level (Al-Ramamneh, 2009). Light stimulated the production of peltateglanulartrichomes, the formation of which is a prerequisite for the accumulation of essential oils in plants. A significant reduction in the essential oil content in chamomile when exposed to pollutants was observed (Razmjoo*et al*., 2008).



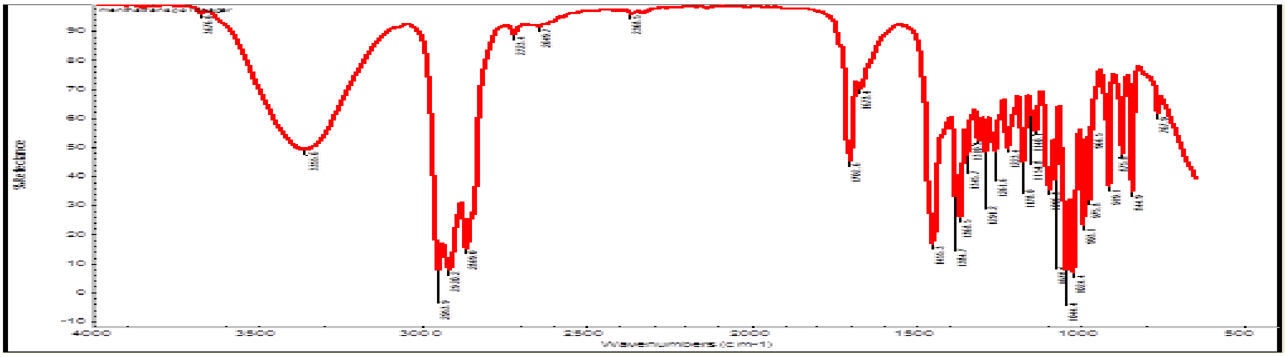
M. arvensis (site 1) university (summer season)



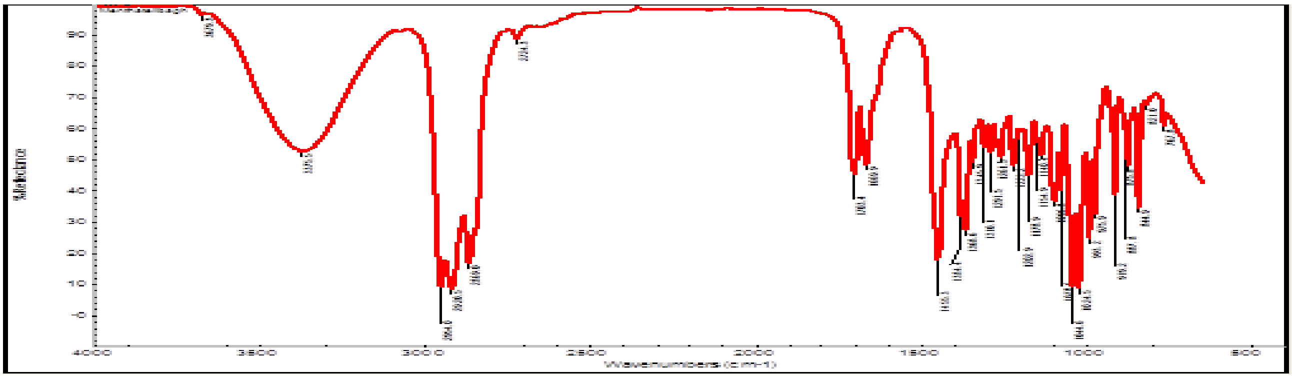
M. arvensis (site2) Hazratganj (summer season)



M. arvensis (site3) charbagh (summer season)

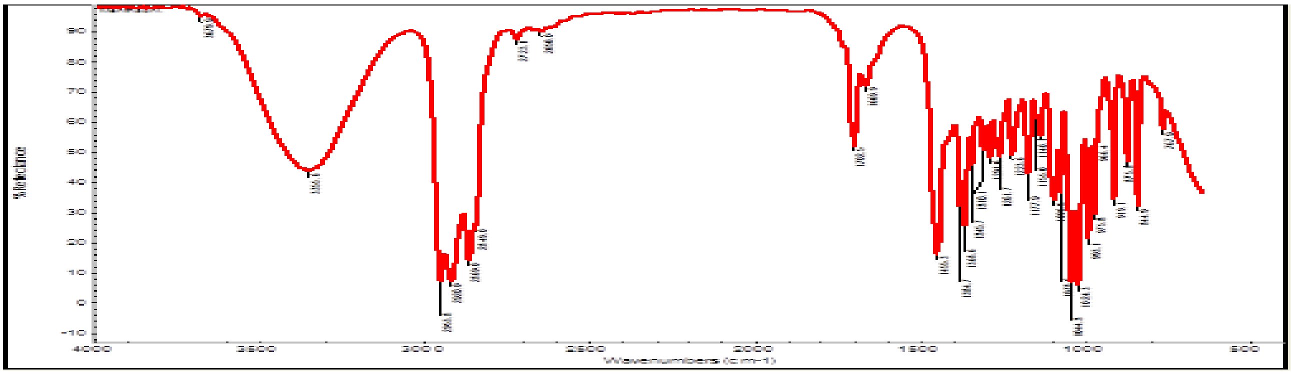


M. arvensis (site 4) transport nagar (summer season)

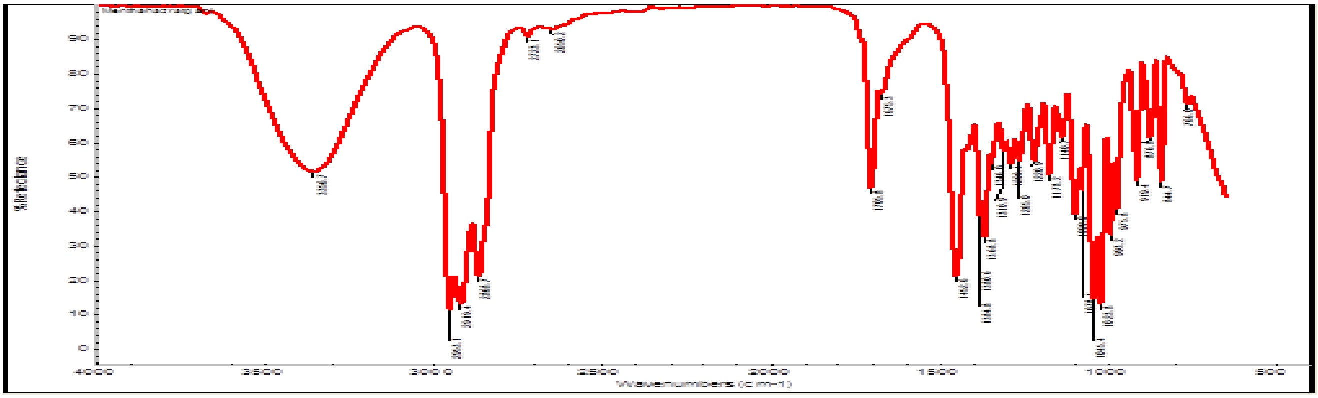


M. arvensis (site 5) telibagh (summer season)

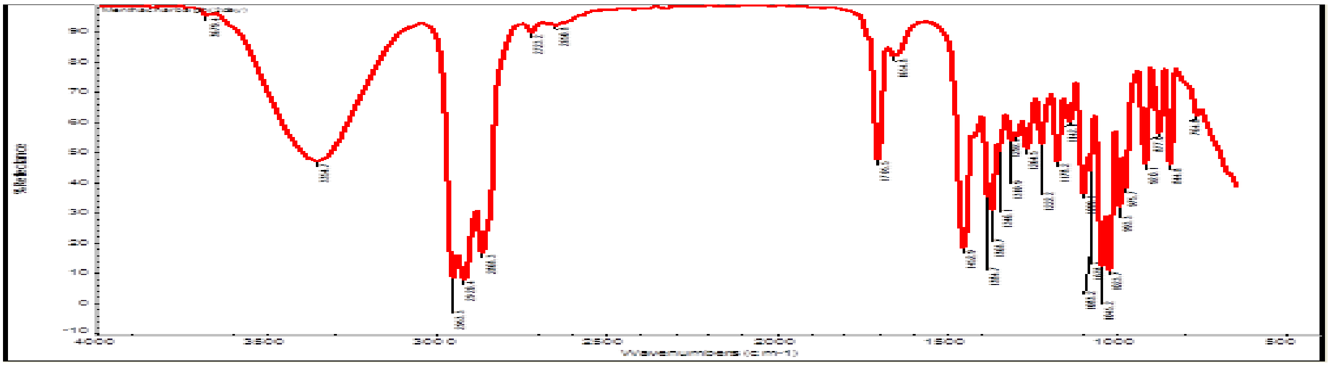
Fig.2. FT-IR Spectra of Oil (*Menthaarvensis, saccham Variety*) samples in summer season from all the selected sites



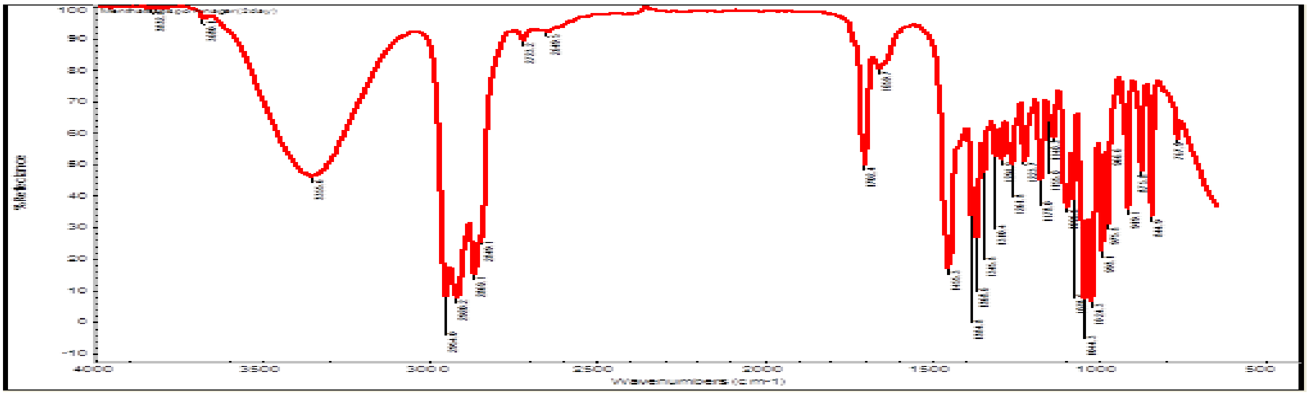
M. arvensis (site 1) university (rainy season



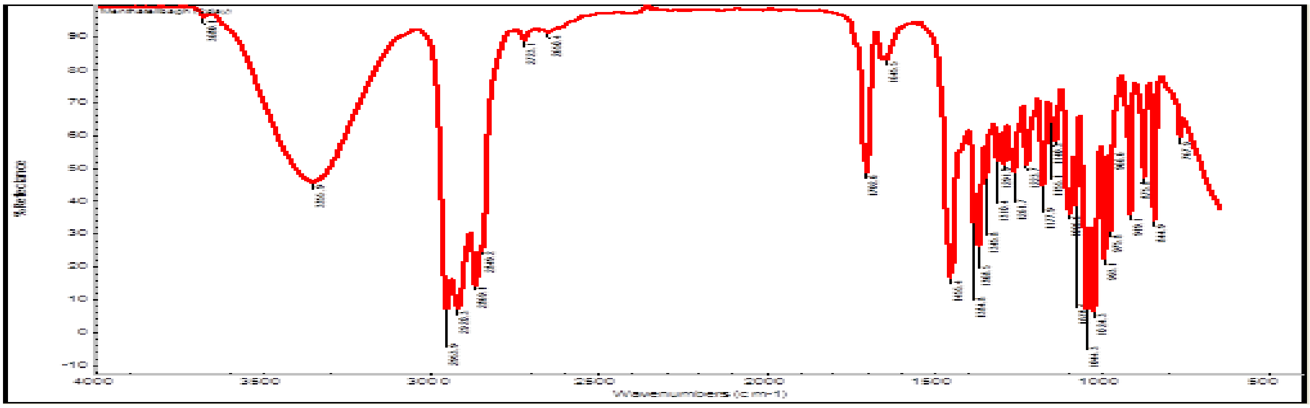
M. arvensis (site2) Hazratganj (rainy season)



M. arvensis (site3) charbagh (rainy season)



M. arvensis (site 4) transport nagar (rainy season)



M. arvensis (site 5) telibagh (rainy season)

Fig.3. FT-IR Spectra of Oil (*Menthaarvensis, saccham Variety*) samples in Rainy season from all the selected sites

Table 3. Assignment of IR absorption bands in the spectra of oil samples (*Menthaarvensis*)

Wavenumbers (cm^{-1})										Tentative assignment
Site 1		Site 2		Site 3		Site 4		Site 5		
Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	
-	-	-	-	-	-	-	3832.0			Acetonitrile , benzene
3679.9	3679.7	3677.9	-	3678.1	3679.4	3676.6	3680.1	3679.3	3680.1	Free O-H group, intermolecular hydrogen bonded (polymeric association)
3355.6	33568	3356.2	3356.7	3355.8	3354.7	3355.6	3355.6	3375.5	3355.9	Intermolecular hydrogen bonded OH, O-H stretching
2953.8 2920.0	2953.9 2920.1	2953.7 2918.6	2953.1 2919.4	2954.0 2920.4	2953.3 2920.4	2953.9 2902.2	2954.0 2920.1	2954.0 2920.5	2953.9 2920.3	Chelate compounds,

										primary alcohols, Aliphatic –CH3 and CH2 Stretching O-H and C-O str
2869.0 2849.0	2869.0 2489.1	2869.1 2847.7	2868.7 -	2869.1 -	2868.3 -	2869.0 -	2869.1 2849.1	2869.0 -	2869.1 2849.2	C-O-CH3 group. C-O str
2723.1	2723.3	2722.7	2723.1	2723.5	2723.2	2734.4	2732.2	2724.3	2723.1	Aldehydes C-H str
2650.0	2650.1	2649.8	2650.2	2649.7	2650.8	2649.7	2649.5	-	2650.4	
1702.5	1702.4	1702.3	1705.8	1702.4	1705.5	1702.6	1702.4	1703.4	1702.6	C=0 stretching vibration, α β unsaturated (aliphatic), di aryl ketones
1669.9	1669.4	1677.1	1675.3	1662.7	1654.8	1673.4	1659.7	1669.9	1645.5	β di ketones enolic) C=O str
1455.3	1455.4	1455.3	1452.6	1455.4	1452.9	1455.3	1455.3	1455.3	1455.4	C=H def of –CH2 orCH3 groups (lignin) in aliphatics
1384.7 1368.6 1345.7 1310.7	1384.6 1368.4 1345.2 1310.6	1384.7 1368.5 1345.7 1310.4	1384.8 1368.8 1346.0 1310.9	1384.7 1368.5 1345.8 1310.7	1384.7 1368.7 1346.1 1310.9	1384.7 1368.5 1345.7 1310.3	1384.8 1368.6 1345.8 1310.4	1384.4 1368.6 1345.9 1310.1	1384.8 1368.5 1345.8 1310.4	Primary alcohols, C-O str
1291.6 1261.7 1223.6 -	1291.4 1261.6 1223.8 -	1290.8 1261.8 1223.2 -	1290.9 1265.0 1220.9 -	1291.6 1261.9 1223.7 -	1292.0 1264.5 1222.2 -	1291.2 1261.6 1223.4 -	1291.9 1261.7 1223.8 -	1291.5 1261.5 1223.2 1202.9	1291.9 1261.7 1223.7 1202.8	Ester carbonyl group, phenol

The IR spectrum of plant samples collected from all sites are shown in **Fig. 2** and **3**. The absorption bands and their tentative assignments are given in Table 2 for the both seasons. The FT-IR spectrum exhibits the characteristic finger print band features. The very strong absorption bands at 2953.9, 1384.5 and 1291 $cm^{–1}$ are representative for C-H, O-H and C-O stretching vibrations, characteristic of the presence of Primary alcohols. In all locations, it is noticed that the bands at 2953, 1702 and 1455 $cm^{–1}$ are due to the stretching vibration of –CH3 and –CH2 groups and aliphatic groups. The 2723, 1669 and 1291 $cm^{–1}$ bands are due to stretching vibration of C-H indicating aldehyde group compounds. The strong bands at 1434 $cm^{–1}$ and 1411 $cm^{–1}$ represent the bending vibrations of CH indicative of the lignins. The 1232 and 1216 $cm^{–1}$ bands in all samples predict the presence of ester carbonyl.

The infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. These differences may be due to the industrial environment. From the spectrum, we can noticed that the bands 3679 $cm^{–1}$ are absent in the samples in rainy season at site 2 but present at all sites in every season .The band 1202.9 cm^{-1}

indicating presence of ester group present only in samples from site 5. The bands 2953 to 2920 cm^{-1} indicating the Chelate compounds, primary alcohols, Aliphatic are strongly present in both Rainy and summer seasons. Aldehyde 2723cm^{-1}, α β unsaturated (aliphatic), di aryl ketones 1702cm-1 and β di ketones enolic groups1669 cm^{-1} are present in the samples in all locations and the absorption is very strong in both seasons. It is also strong in the control sample. Likewise the lignins are present in the sample from strong absorption to weak absorption whereas it has medium absorption in the control sample. The band at 2951 and 2920 cm-1 are due to the C-H asymmetric and symmetric stretching of saturated (sp3) carbon, respectively. The band at 1667 cm-1 is assigned to the bending mode of absorbed water, since plant extracts are known to have a strong affinity for water. From the above discussion it results that the bands 3679 cm^{-1} (O-H), 3355 cm^{-1} ((O-H), 2953 cm^{-1} (CH3CH2),2869 cm^{-1} (C-O C-H3), 2723 cm^{-1} (C-H) and 1702 cm^{-1}(C=O),1455 cm^{-1}(C=H, -CH2 CH3),1384 cm^{-1} (C-O primary alcohols) and 1291cm^{-1}(ester carbonyl group), confirm the presence of Menthol and menthonone main constituents of Menthol mint oil. It was found that the Oil contains all organic compounds: primary alcohols, enolic compounds, ester groups, aliphatic compounds, lignins etc. in the oil from the control site. It was also observed that some of the compounds are missing or some compounds are also added iin oil from other polluted sites.

Conclusion

Mint species are used widely throughout the world as an important medicinal plant. Their oils are one of the most popular and widely used essential oils, mostly because of its main components such as menthol and isomenthonone. Essential oils and oxygen pass through the capillary walls and into the bloodstream. Most of the essential oils are absorbed into the cells lining the respiratory passages and this may be an indicator why they work well on respiratory ailments. Some of them pass with oxygen into blood capillaries where they enter into blood circulation. Essential oils are easily absorbed and can diffuse throughout the body.

From this analysis, we may conclude that only some changes have occurred in chemical constituents of oil from *Menthaarvensis*. The plant is exposed to the pollution of vehicles all the time, only minor changes in its constituents are noticed which suggests that this plant has a protective mechanism but it's not totally safe to consume, because the oil is widely used at very large scale in food industries. So do not grow or plant Menthol mint plant in the field nearby highways or railways tracks.

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