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ORIGINAL RESEARCH ARTICLE

Allelopathy as a tool to influence essential oil quality profile of *Mentha piperita* L.

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ABSTRACT

The present investigation focuses on the prospect of using allelopathy as a tool to alter the essential oil profile of Mentha piperita L. For this, the allelopathic influence of Rauvolfia tetraphylla L. (Apocynaceae) on Mentha piperita L. (Lamiaceae) crop was investigated. The study reveals that menthyl acetate and menthofuran components exhibit increasing trend while menthone, iso-menthone and pulegone components of the essential oil of Mentha piperita L. exhibits decreasing trend. Five fold increase in the concentration of menthyl acetate was also evident when it is grown along with Rauvolfia tetraphylla L.

INTRODUCTION

Allelopathy is an interesting phenomena/ mechanism of plant interference mediated by the release of plant-produced metabolites or decomposition products of microbes in the aerial or soil environment in natural as well as cultivated ecosystems. Allelochemicals are released into the soil rhizosphere by many ways like volatilization, decomposition of residues and root exudation. A number of plant organs may be instrumental in exhibiting allelopathic interaction like foliage, flowers, roots, bark and mulch. Defoliation is considered to be the most common form of this activity as leaves fall to the ground, decompose and release allelochemicals in the soil. The allelochemicals spread along the soil to affect nearby plants. Trees too exhibit allelopathy to protect their space by pulling more water from the soil, so that other competing plants do not thrive nearby. Other allelopathic plants channel their allelochemicals to impede growth and development of nearby competing plants.

The potential impacts of using the benefits of allelopathy on agricultural perspective have been studied and exploited in detail (Putnam and Duke, 1978; Putnam, 1986; Putnam and Weston, 1986; Qasem and Foy, 2001; Rice, 1984; Singh et al., 2001; Weston, 1996). Putnam and Duke (Putnam and Duke, 1974) first explored the possibility of using crops having allelopathic effect to suppress weeds in agricultural systems. This paved way for developing weed-suppressive crops as rotational, inter, or cover crops for effective weed suppression. Classical breeding methods have however not been employed to produce effective allelopathic crops with end benefits may be because of multigenic

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regulation of allelochemical biosynthesis. Early allelopathic research was mainly focused on the detrimental effects of allelopathic plants or their residues on of growth and yields of cultivated crops. In other words the paradigm was majorly concentrated on the negative effects or non-beneficial attributes of allelopathy. But now the negative effect concept is observing a paradigm shift and allelopathy today is being exploited as a contributor in enhancing secondary metabolite profile of economically important aromatic crops. Most recently, the number of publications in the field of allelopathy has increased exponentially as physiologists, soil scientists, weed scientists and natural products chemists continue to study this challenging area (Begum et al., 2020; Isin Ozkan et al., 2019; Pula et al., 2020; Liu et al., 2020; Macias, 2002). The present investigation was carried out to determine whether *Rauvolfia tetraphylla* L. can exhibit allelopathic effect on *Mentha piperita* L in a co-cultivation condition (Fig. 1), thereby exploring the possibility of exploiting the phenomena of allelopathy in relation to the alteration of secondary metabolite profile in essential oil of *Mentha piperita* L.

MATERIALS AND METHODS

Plant materials

Rauvolfia tetraphylla L., a member of Apocynaceae family is a bush or small tree. It has been cultivated widely, both as an ornamental plant and for use in traditional medicine. It contains indole alkaloids like serpentine, reserpine, serpentinine etc.

Mentha piperita L. commonly called as peppermint is a member of Lamiaceae family. It is a herbaceous, rhizomatous, perennial plant that grows to a height of 30–90 cm and has smooth stems. The rhizomes bear fibrous roots. The leaves are 4–5 cm long and 1.5–3 cm broad. They are dark green with reddish veins, have an acute apex and coarsely toothed margins. This plant is a source of menthyl acetate, menthofuran, menthone, iso-menthone, pulegone, menthol (Fig. 2) etc. It is among the oldest herbs used for both culinary and medicinal products.

Both the plants were available at the Research Station, Chatha, CSIR-Indian Institute of Integrative Medicine (IIIM), Jammu (India).

Field preparation and allied practices

The field experiment was laid down in a randomized complete block design with ten replicates and two treatments (check and experiment), having plot size 2.56m^2 at Chatha Field Research Station of CSIR –Indian Institute of Integrative Medicine (IIIM), Jammu (India) located at 32°39'40" (N) to 32°40'00" (N) latitude and 74°48'40" (E) to 74°49'10" (E) longitude (Fig. 3). The altitude of the area is 300m (approx.) above sea level. The annual precipitation at this site is 500–700 mm. The climate was semiarid to subtropical in nature. Minimum and maximum night and day temperatures ranged 6–11°C and 15–17°C, respectively during growth period and, from 25–30°C and 35–40°C during harvesting time, respectively. The crop received normal intercultural operations, irrigations and fertilizer applications (60kg N, 30kg P_2O_5 and 30kg K_2O per hectare).

Statistical analysis

Morphometric observations were recorded using ten randomly chosen plants from each treatment with respect to six important yield contributing characters namely: plant height (the length of the erect plants from the ground surface to the shoot apex was measured in centimeters), number of branches (total number of branches present in a plant), leaf length (length from the apex to the base of lamina measured in centimeters), leaf breadth (measurement of the widest part of the lamina in centimeters), fresh weight (weight of the freshly harvested biomass in grams) and oil yield (after hydro-distillation, the amount of oil in milliliters obtained from a gram of freshly harvested biomass). The data for all the above traits were collected in two time intervals – after 95 days and after 125 days of plantation. The mean data was subjected to statistical analysis using statistical software 3.0 version available at the Department of Genetics and Plant Breeding of the Institute.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of each sample was carried out on Agilent (S-80) series GC-MS equipped with a Combi Pal auto-sampler. The columns used were Agilent J&W DB-5 GC capillary column (30 m × 0.25 mm i.d., 0.25 µm) DB-5. Helium was used as the carrier gas. The column temperature was initially programmed at 60°C, held for 5 min and increased to 280°C at 10°C/min for 27 minutes through splitless mode. Injector and detector temperatures were 250°C. The ionization energy was 70eV and a mass range of 45–500 AMU. The management of the GC-MS system, parameter settings for GC and mass spectrometry, data acquisition and processing were performed using Agilent Chemstation. The compounds were identified using NIST library.

RESULTS

ANOVA represents highly significant ($p < 0.01$) differences for the traits plant height (cm), leaf breadth (cm) and fresh weight (g) while significant ($p < 0.05$) differences appear in number of branches, leaf length(cm) and oil yield (ml/g) as evident from Table 1.

GC-MS results after 95 days of growth indicated the presence of 1.87% menthyl acetate in check, 10.26% menthyl acetate in experiments, 22.36% menthofuran in check and 26.02% menthofuran in experiments (Table 2, Fig. 4). In addition, 23.98% menthone in check, 8.34% menthone in experiments, 4.07% iso-menthone in check, 1.40% iso-menthone in experiments and 12.13% pulegone in check and 11.26% pulegone in experiments were also detected (Table 2, Fig. 4). Menthol was detected neither in check nor in experiments as evident from Table 2.

GC-MS results after one hundred and twenty five days of growth indicated the presence of 2.58% menthyl acetate in check, 9.75% menthyl acetate in experiments, 15.44% menthofuran in check and 20.65% menthofuran in experiments (Table 2, Figs. 5, 6 and 7). Other chemical constitutents detected were as follows: 15.13% menthone in check, no menthone in experiments, 10.11% iso-menthone in check, 9.13% iso-menthone in experiments, 12.17% pulegone in check 9.50% pulegone in experiments, 15.47% menthol in check and 14.81% menthol in experiments (Table 2, Fig. 5).

Table 1: ANOVA for six traits of *Mentha piperita L.*

Sl No	Traits	Replications (MS) df=9	Treatments (MS) df=1	Error (MS) df=9
1	Plant Height (cm)	7.12	245.00**	2.69
2	Branches	0.69	0.80*	0.13
3	Leaf length(cm)	0.005	0.06*	0.01
4	Leaf breadth(cm)	0.02	0.20**	0.01
5	Fresh weight(g)	4.61	143.11**	2.89
6	Oil yield(ml/g)	0.002	0.013*	0.002

Where, * = $p < 0.05$; ** = $p < 0.01$, respectively

Table 2: Variation in essential oil concentration as per GCMS analysis

Sl no	Compounds with a difference in concentration	Observation after 95 days		Observation after 125 days	
		Check (%)	Experiment (%)	Check (%)	Experiment (%)
1	Menthone	23.98	8.34	15.13	Not detected
2	Isomenthone	4.07	1.40	10.11	9.13
3	Menthyl acetate	1.87	10.26	2.58	9.75
4	Menthofuran	22.36	26.02	15.44	20.65
5	Pulegone	12.13	11.26	12.17	9.504
6	Menthol	Not detected	Not detected	15.47	14.81

Figure 1: (a) *Mentha piperita* L. (check) growing alone; (b) *Mentha piperita* L. and *Rauvolfia tetraphylla* L. growing in co-cultivation state

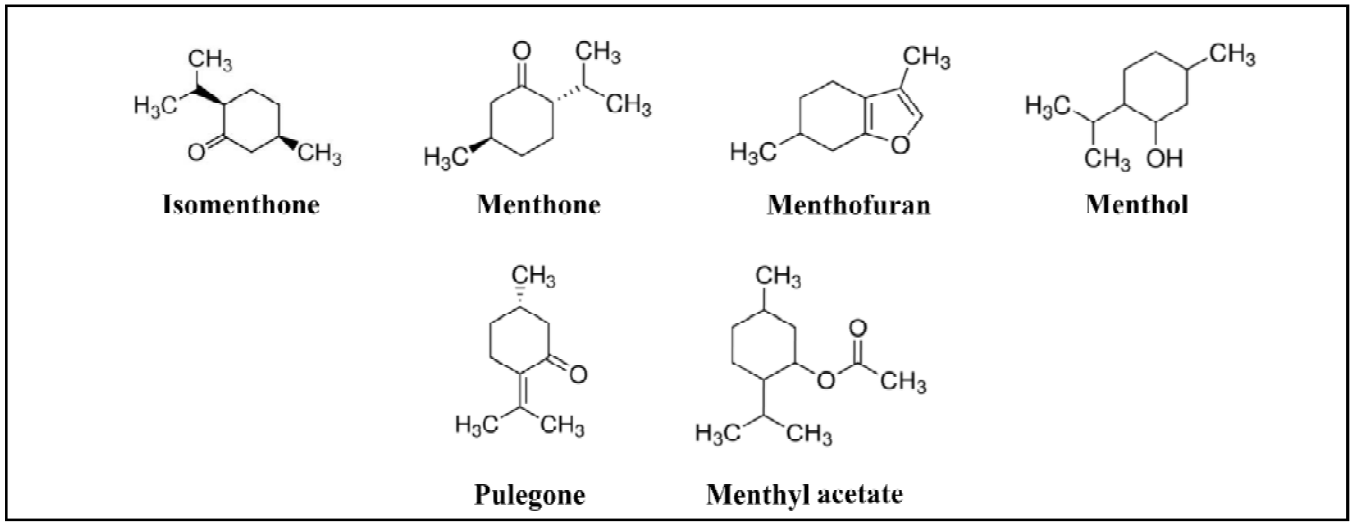

Figure 2: Structures of chemical components identified in GC-MS

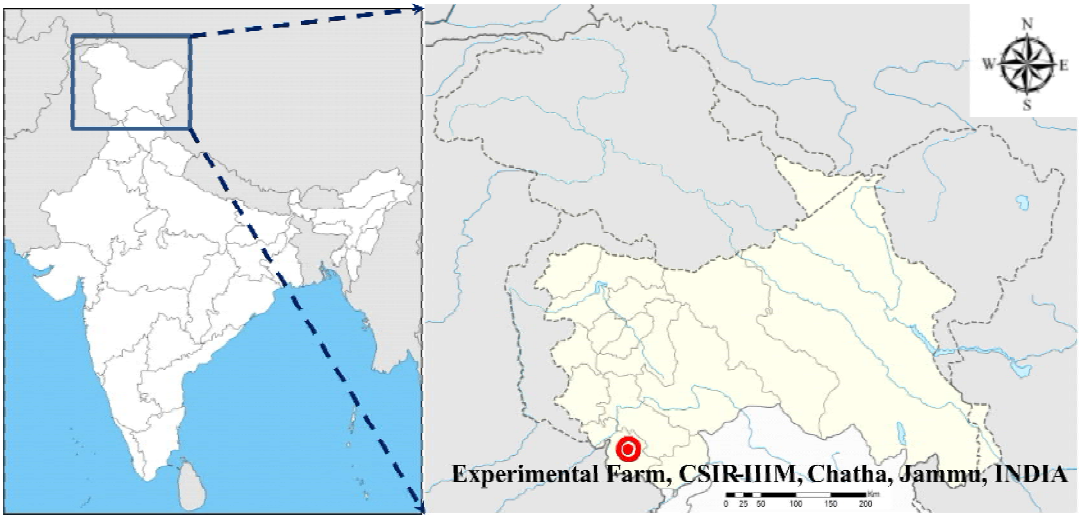

Figure 3: Site of experimental farm

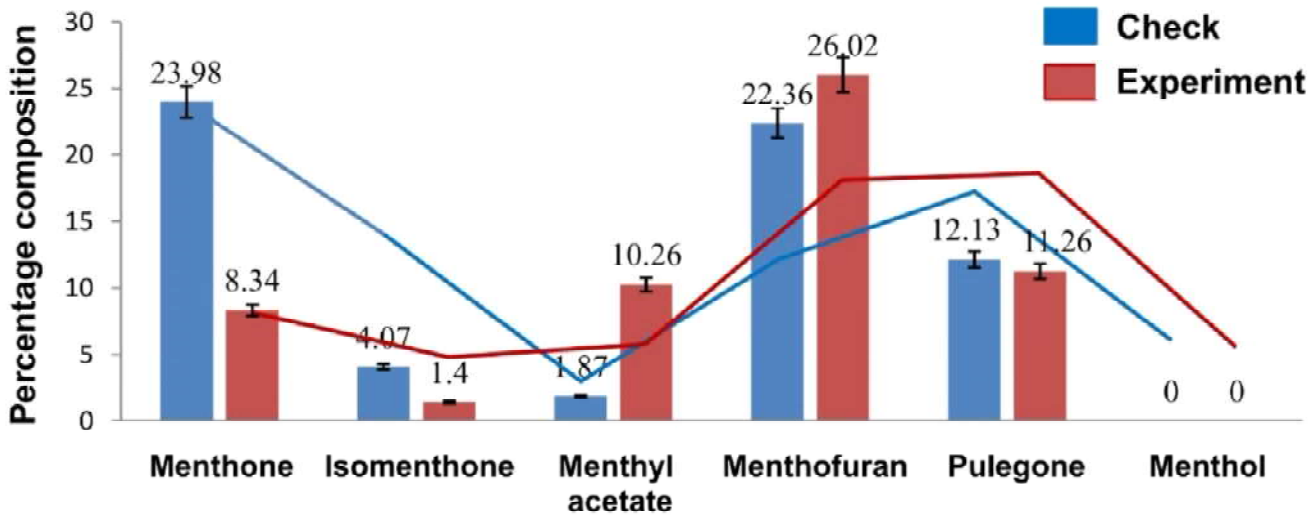

Figure 4: Components of essential oil of *Mentha piperita* after 95 days of planting

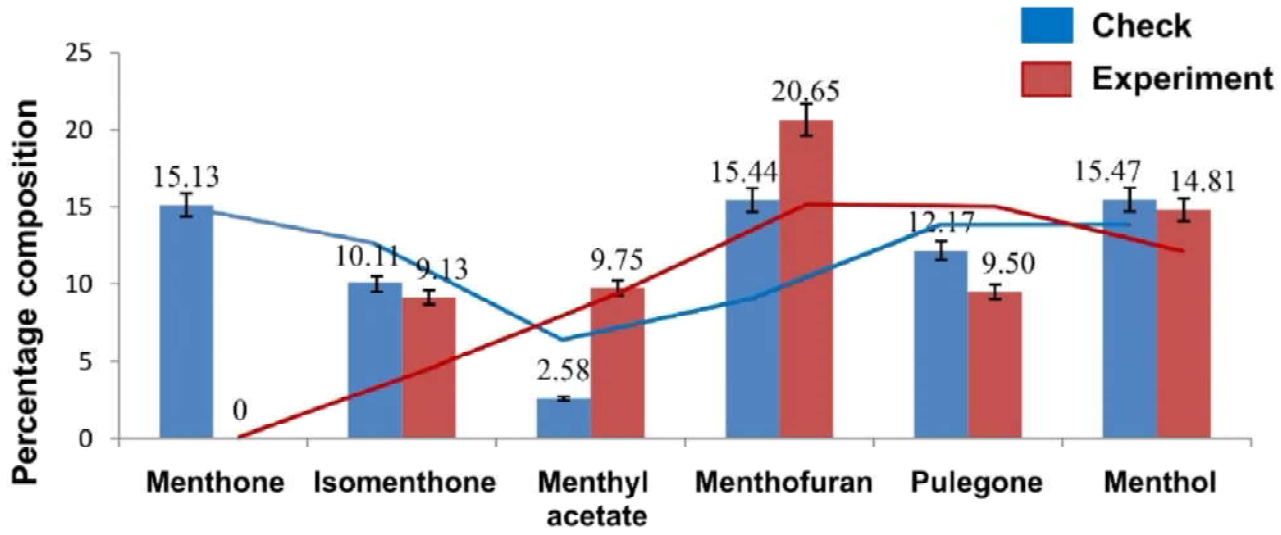

Figure 5: Components of essential oil of *Mentha piperita* after 125 days of planting

DISCUSSION

Menthyl acetate and menthofuran components of essential oil in *Mentha piperita* L. exhibited increasing trend in check as well as in experiments at both time intervals of 95 days and 125 days. After a span of 95 days, the difference in concentration of menthyl acetate and menthofuran in check and experiment were recorded as 8.39% and 3.66% respectively, while the corresponding values recorded after a span of 125 days, were 7.17% and 5.21%, respectively. This reflects that production of menthyl acetate is diminished by 1.22% whereas the production of menthofuran is enhanced by 1.55% during the span of 30 days. Menthone and pulegone components exhibited decreasing trend in check as well as in experiments in both time intervals of 95 and 125 days. The concentration of iso-menthone during 125 days and menthol during both the time intervals of 95 and 125 days remained almost unaffected. After a span of 125 days, the difference in concentration of iso-menthone in check (10.11%) and experiment (9.13%) was recorded to be less than 1% (0.98%) whereas after the same span of time, concentration of menthol

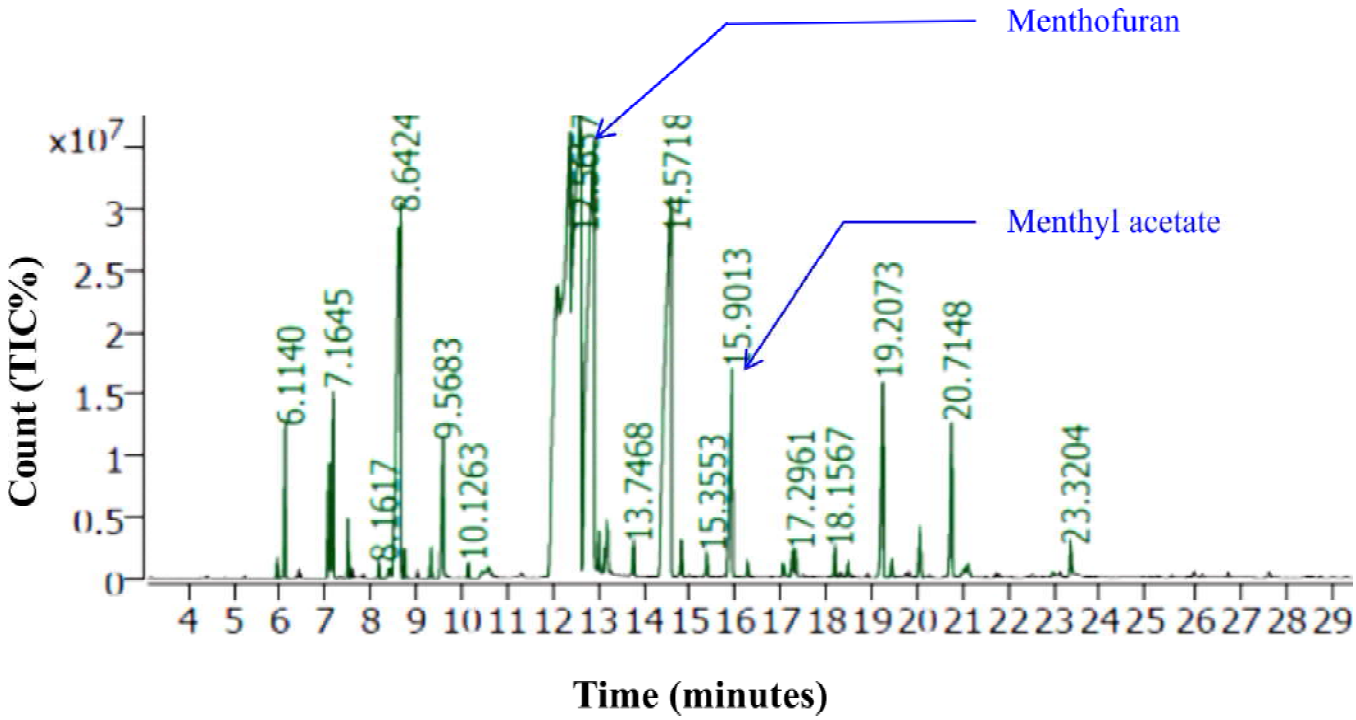

Figure 6: GC-MS chromatogram of menthyl acetate and menthofuran components of *Mentha piperita* (check) after 125 days of planting

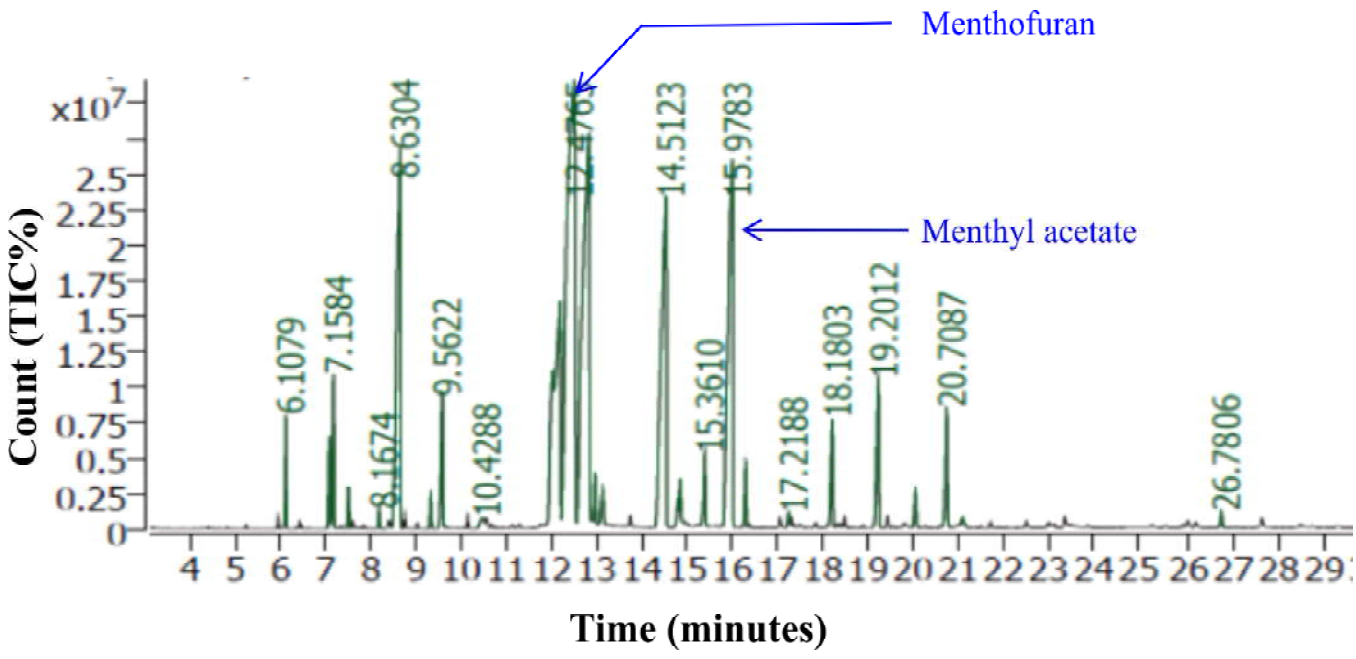

Figure 7: GC-MS chromatogram of menthyl acetate and menthofuran components of *Mentha piperita* (experiment) after 125 days of planting

in check (15.47%) and experiment (14.81%) was also recorded to be less than 1% (0.66%). Menthol component could not be detected till the initial 95 days of growth period both in check and experiment whereas menthone component in experiment could not be detected after 125 days of initial growth period. From the GC-MS results, it may be ascertained that the biochemical process of formation of menthol took more than 95 days to activate itself in *Mentha piperita* L. It is interesting to deduce that menthone component may have uniformly given way to its downstream components/products in check and experiments soon after 95 days of growth period, which exhibits

harmonious reduction in yield of this component after 125 days.

Mentha piperita L., being an export oriented crop of India, has a considerable indigenous and foreign market. The valuation of menthyl acetate component depends on its physical form (natural solid form, liquid form and 100% pure form) in the indigenous market. Import of menthyl acetate was to the tune of 3380kg/year from Germany and Spain amounting to $56,979.00 whereas export was to the tune of 153,707kg/year to USA ($1,983,876.00), China ($466180.00) and Germany ($156,790.00) amounting to a total of $3,007,535.00 in the year 2016.

CONCLUSION

The study revealed that menthyl acetate and menthofuran components exhibited increasing trend while menthone, iso-menthone and pulegone components exhibited decreasing trend in the essential oil profile of *Mentha piperita* L., when it is cocultivated with *R. tetraphylla* L. It was interesting to note that a little more than five fold increase (i.e. from 1.87% to 10.26%) was observed in the concentration of menthyl acetate after imposing the phenomenon of allelopathy which had contributed positively to the enhancement of menthyl acetate component in the essential oil of *Mentha piperita* L. The period between ninety five days to one hundred twenty five days after plantation proved to be the best time for harvesting in order to get maximum menthyl acetate from the essential oil of *Mentha piperita* L. when it is grown along with *R. tetraphylla* L.

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