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ORIGINAL RESEARCH ARTICLE FOR VARIETY REGISTRATION

CIM-Unnati: An essential oil rich variety of menthol mint (*Mentha arvensis* L.)

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ABSTRACT

Menthol mint (Mentha arvensis L.) is cultivated commercially for extraction of essential oil from upper ground herbage (leaves + stem). The essential oil of menthol mint is mainly used for production of menthol and the by-product dementholised oil (DMO) that find extensive applications in pharmaceutical and cosmetic industry. A new genotype of menthol mint MASP-19, now christened as CIM-Unnati, has been developed by CSIR-CIMAP through intensive breeding efforts for improved essential oil yield. The new variety CIM-Unnati consistently recorded higher essential oil yield over the commercial checks during field evaluation trials. The new variety has a potential to produce 185-190 q/ha herbage that can give an essential oil yield of 185-190 litre/ha. The variety matures in 100-110 days after planting. The menthol content in the oil is hovers around 74.3 %, which is as per industry requirement. The distinct leaf morphology and high essential oil yield are the two important features of this variety. This variety also satisfies several DUS (Distinctness, Uniformity and Stability) criteria.

INTRODUCTION

Menthol mint (*Mentha arvensis* L.) belonging to the family Lamiaceae, is an important medicinal and aromatic crop of the sub-tropical countries, including India. The other major menthol mint producing countries in the world are USA, several European countries, China and Brazil (Venkatesha et al., 2019). In India, menthol-mint is cultivated in more than 200,000 ha area in Uttar Pradesh, Bihar, Haryana, Punjab, West Bengal, Jammu & Kashmir, Himachal Pradesh and Uttarakhand (Rao and Syamasundar, 2013; Shukla et al., 2011). The essential oil of menthol mint is commercially used for producing menthol and the by-product dementholised oil (DMO) that find wide use in pharmaceutical and cosmetic industry. Menthol has a cooling and soothing effect on the skin, making it a useful ingredient in several pharmaceutical and

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cosmetic products available in the market. It is also commercially used in oral care products, tobacco products, confectionaries, chewing gum, perfume, and lotions (Venkatesha et al., 2019).

India is at present the leading producer, consumer and exporter of menthol-mint oil in the world market with more than 80% of global share due to suitable agro-climatic conditions and availability of several improved varieties (Padalia et al., 2015; Singh and Khanuja, 2007). The high price of menthol mint oil in the national and international markets, following the drop in supply of its synthetic substitute, has propelled new hope among the menthol mint cultivating farmers (Singh and Khanuja, 2007). Presently, cultivated menthol mint varieties have moderate oil yielding potential of 50-60 kg/acre. Keeping all this in mind, a need was felt to develop a better plant type having high essential oil yielding capacity. Hence, a planned menthol mint breeding program was undertaken at CSIR-CIMAP, Research Centre, Pantnagar, Uttarakhand to develop a new variety of menthol mint.

MATERIALS AND METHODS

Under a genetic improvement program on essential oil crops, genetic improvement of menthol mint for high herb and high oil yield was undertaken at CSIR-CIMAP, Research Centre, Pantnagar, in 2016. Menthol mint is a highly cross pollinated plant and its flower size is very small, so it is very difficult to practice emasculation and pollinations to produce hybrids (F_1s). Also it is very difficult to produce selfed seed progenies because under selfing it does not produce seeds. So, it is very difficult to produce any transgressive segregants

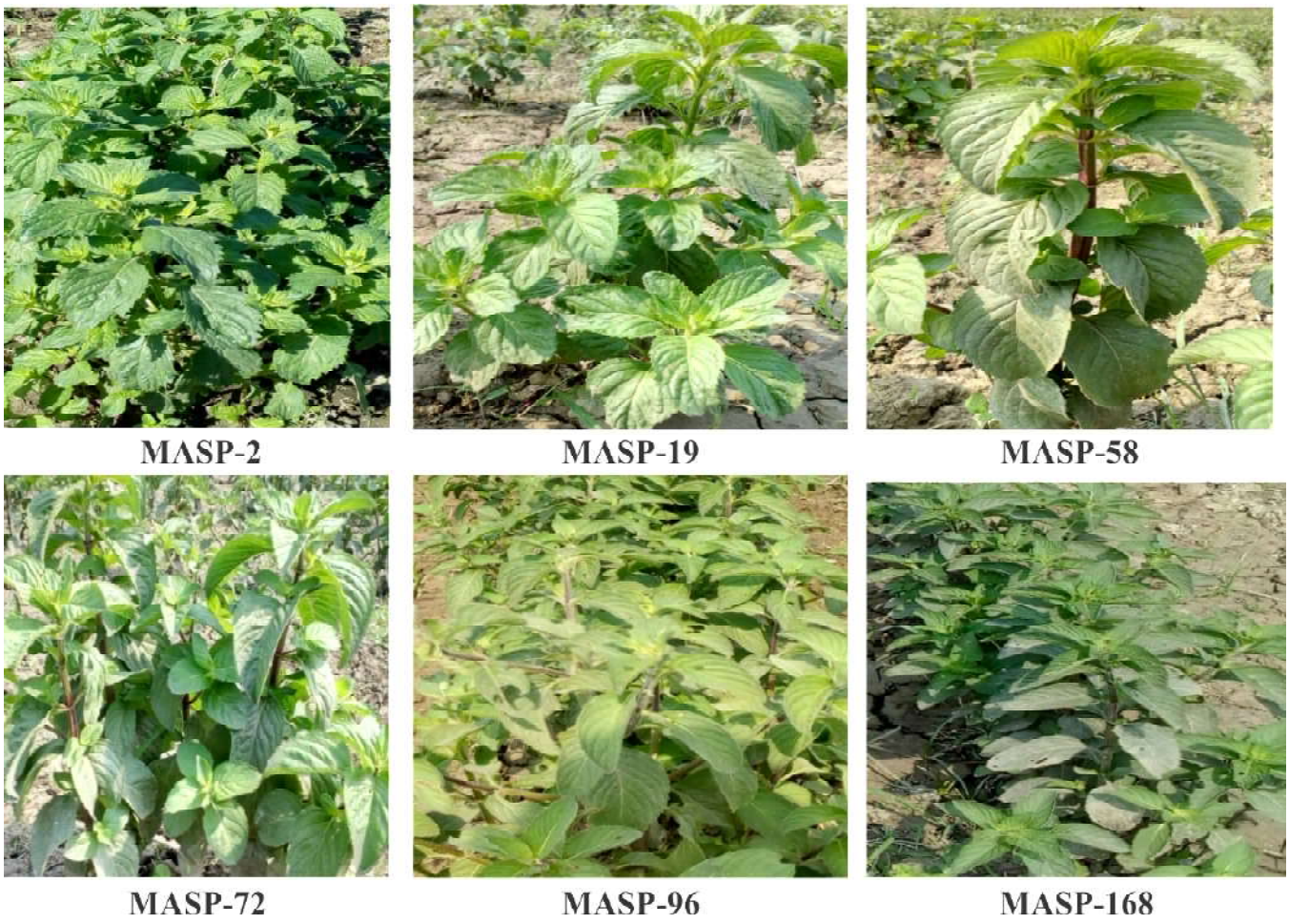

Figure 2: Morphologically distinct half-sib progenies recovered

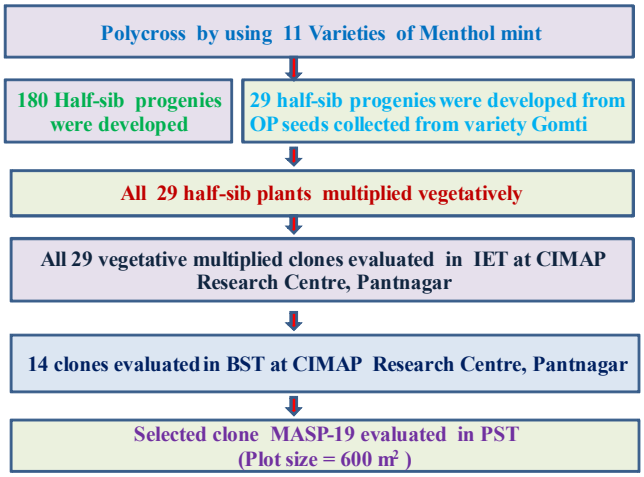

Figure 1: Breeding history of the newly developed variety CIM-Unnati (MASP-19)

in F_2 and subsequent segregating generation. Therefore a population improvement method (half-sib progeny selection) was applied by using 11 menthol mint varieties released by CSIR-CIMAP namely, Kalka, Saksham, Himalaya, CIM-Saryu, Gomti, Damroo, Kushal, Kosi, CIM-Kranti, MAS-1 and Sambhav. All these 11 varieties were planted side-by-side and allowed to flower. During flowering, natural random mating was allowed among them and after maturity seeds were collected from all the 11 maternal parents, separately (Fig. 1).

Table 1: Mean performance of 29 half-sib clones of menthol mint in IET (Entries=31, Field design= RBD, Replications = 3, Plot size = 9m²)

Accessions	Plant height (cm)	Canopy width (cm)	Herb yield /plot (kg)	Oil percent (%)	Oil yield/ plot (g)
MASP-1	115.00	37.50	12.90	0.50	64.50
MASP-2	118.00	40.00	15.90	1.30	206.70
MASP-3	98.00	39.00	13.35	0.60	80.10
MASP-4	94.00	40.00	13.60	0.55	74.80
MASP-5	105.00	32.00	14.70	0.65	95.55
MASP-6	117.00	47.50	14.30	0.60	85.80
MASP-7	106.00	46.00	14.60	0.70	102.20
MASP-8	108.00	43.00	14.70	0.85	124.95
MASP-9	94.00	43.50	13.55	0.90	121.95
MASP-10	104.00	42.50	14.40	0.80	115.20
MASP-11	98.00	30.50	14.90	0.85	126.65
MASP-12	112.00	30.84	13.15	0.85	111.78
MASP-13	106.00	34.00	13.60	0.80	108.80
MASP-14	128.00	48.50	15.05	0.82	123.41
MASP-15	122.00	38.00	13.90	0.80	111.20
MASP-16	109.00	29.00	14.30	1.00	143.00
MASP-17	114.00	50.00	13.10	0.85	111.35
MASP-18	118.00	40.00	13.65	0.80	109.20
MASP-19	121.00	46.00	16.45	1.40	230.30
MASP-20	102.00	55.00	13.10	0.70	91.70
MASP-21	98.00	28.50	13.95	0.85	118.58
MASP-22	93.00	43.00	13.20	0.81	106.92
MASP-23	112.00	40.00	14.20	0.80	113.60
MASP-24	98.00	33.50	13.65	0.67	91.46
MASP-25	97.00	32.00	14.15	0.85	120.28
MASP-26	112.00	42.00	14.45	0.80	115.60
MASP-27	119.00	39.00	13.70	0.75	102.75
MASP-28	107.00	38.00	13.95	0.80	111.60
MASP-29	109.00	43.00	14.20	0.80	113.60
Kosi (Check)	120.00	38.00	16.00	1.00	160.00
CIM-Kranti (Check)	128.00	44.00	16.75	0.90	150.75
Range	93.00-128.00	28.50-55.00	12.90-16.75	0.50-1.40	64.50-230.30
CV	0.58	1.47	0.82	0.23	0.21
CD 5%	1.31	1.22	0.22	0.004	0.48
CD 1%	1.78	1.63	0.31	0.005	0.64

A total of 180 half-sib progenies/seed progenies were developed by using seeds collected from all the 11 maternal parents. Out of these 180 half-sib progenies, 29 were obtained from the open pollinated seeds (half-sibs) collected from the variety Gomti (as maternal parent). Among these 29 half-sibs, some half-sibs were morphologically distinct and had high essential oil content (Fig 2). Hence, we intentionally selected all the 29 half-sibs for further study. The vegetatively-multiplied half-sib progenies (MASP-1 to MASP-29) were first evaluated in an Initial Evaluation Trial (Plot size = 9 m^2; RBD Design; Replications 3; row to row spacing 60 cm; plant to plant 30 cm) for their agronomic performance against the check varieties, Kosi and CIM-Kranti (Table 1).

RESULTS AND DISCUSSION

Based on the results of the IET, out of 29 vegetatively propagated half-sib progenies, 14 superior clones were selected on the basis of their essential oil yield. These 14 clones were further evaluated for agronomic traits and chemical constituents of their essential oil in a Bench Scale Trial (BST) along with check varieties, Kosi and CIM-Kranti (Entries=14+2 checks, RBD, Replications=3, Plot size=9 m^2). Results are summarized in Table 2. In BST, the best performing clone MASP-19 was further selected on the basis of essential oil yield was evaluated in a Pilot Scale Trial (PST); (Plot size 600 m^2) with check varieties, Kosi and CIM-Kranti (Table 3). Throughout the study, the clone MASP-19 maintained its superiority over the checks for essential oil yield (Tables 1-3). This high essential oil yielding clone MASP-19, now christened as CIM-Unnati, (Fig. 3) is hereby proposed to be registered for its release as an improved cultivar for commercial cultivation.

Figure 3: Field view of the essential oil rich variety CIM-Unnati of menthol mint (*Mentha arvensis* L.)

Table 2: Mean performance of selected clones of menthol mint in BST (Entries=16, Field design= RBD, Replications = 3, Plot size= 9m^2)

Accessions	Plant height (cm)	Canopy width (cm)	Leaf: Stem ratio	Herb yield /plot (kg)	Estimated herb yield/ha (q)	Oil percent (%)	Oil yield/plot (g)	Estimated oil yield/ha(kg)
MASP-2	123.20	42.00	1.30	16.00	177.78	1.30	208.00	231.11
MASP-8	115.60	41.00	0.80	14.90	165.55	0.80	119.20	132.44
MASP-9	105.20	45.00	0.70	14.30	158.89	0.80	114.40	127.11
MASP-10	109.40	44.10	0.90	14.80	164.44	0.90	133.20	148.00
MASP-11	106.10	30.50	0.80	15.20	168.89	0.80	121.60	135.11
MASP-12	119.80	30.84	0.60	13.15	146.11	0.80	105.20	116.89
MASP-14	121.30	52.40	0.80	15.40	171.11	0.80	123.20	136.89
MASP-16	105.40	38.20	0.90	14.85	165.00	0.90	133.65	148.50
MASP-17	116.80	50.10	0.80	14.20	157.78	0.85	120.70	134.11
MASP-19	126.50	46.80	1.40	16.35	181.66	1.40	228.90	254.33
MASP-21	97.90	34.20	0.80	14.30	158.89	0.86	122.98	136.64
MASP-25	104.50	38.40	0.70	14.95	166.11	0.82	122.59	136.21
MASP-26	121.50	44.60	0.80	14.20	157.78	0.80	113.60	126.22
MASP-29	114.30	45.70	0.80	14.60	162.22	0.76	110.96	123.29
Kosi (Check)	123.70	41.10	1.30	16.15	179.44	1.10	177.65	197.39
CIM-Kranti (Check)	131.80	45.20	1.10	16.70	185.55	1.00	167.00	185.55
Range	97.90- 131.80	30.50-52.40	0.60-1.40	13.15-16.70	146.11-185.55	0.76-1.40	105.20-228.90	116.89-254.33
CV	0.15	0.49	0.84	0.84	185.27	0.54	13.02	0.040
CD 5%	0.37	0.43	0.016	0.26	0.33	0.010	37.68	0.13
CD 1%	0.51	0.60	0.022	0.37	0.45	0.014	52.09	0.18

Table 3: Mean performance of clone MASP-19 in PST (Plot size = 600 m²)

Attribute	MASP-19	Kosi (Check)	CIM-Kranti (Check)
Plant height (cm)	125.40	120.10	135.50
Canopy width (cm)	44.30	40.40	48.20
Herb yield/plot (q)	12.34	12.45	12.68
Leaf: Stem ratio	1.40	1.30	1.10
Estimated herb yield/ha (q)	185-190	191.66	194.44
Oil (%)	1.0	0.85	0.80
Estimated oil yield/ha (L/ha)	185-190	162.92	155.55
Improvement of oil yield over Kosi (%)	14.23		
Improvement of oil yield over CIM-Kranti (%)	19.64		

Table 4: DUS characters of MASP-19 in comparison to varieties, Kosi and CIM-Kranti

Attribute	MASP-19	Kosi (Check)	CIM-Kranti (Check)
Growth habit	Bushy	Globular shape	Open
Plant height (cm)	125.40	120.10	135.50
Canopy width (cm)	44.30	40.40	48.20
Stem	Quadrangular, hard, light green	Quadrangular, yellowish green	Quadrangular, light purple
Length of internodes (cm)	3.2 to 3.9	3.5 to 4.0	4.0 to 4.5
Leaf color	Light green	Light green	Dark green
Leaf arrangement	Opposite	Opposite	Opposite
Leaf texture	Thick and rigid	Moderately thick and rigid	Thick and moderately rigid
Leaf surface	Moderately hairy	Moderately hairy	Moderately hairy
Leaf margin	Deep serration	Shallow serration	Shallow serration
Mean Leaf length (cm)	6.3	8.0	7.2
Mean Leaf width (cm)	4.5	3.0	4.2
Mean Petiole length (cm)	1.3	1.0-1.5	1.4

Statement of distinction/Breeder's claim

The variety CIM-Unnati is an erect plant type with light green leaves having deep serrations in the margin. Its regular and symmetric growth habit results in a globular plant canopy. The stem is also light green in color. The DUS characteristic attributes of the new variety are summarized in Table 4.

DNA profile of new menthol mint variety CIM-Unnati (MASP-19)

For IPR protection, DNA finger printing of CIM-Unnati was carried out. For this, the DNA isolation was carried out from the plant leaves by either using Plant DNAzol™ reagent (ThermoFisher) or DNeasy® Plant Mini Kit (Qiagen) as per the manufacturer's protocol and its quantification was done using a Nanodrop ND-1000 spectro-photometer. Polymerase chain reactions (PCRs) for DNA profiling were carried out in 50 µl. Each reaction contained 50 ng DNA, 1X PCR reaction buffer, 1.5 U *Taq* DNA polymerase (GeNei, Bangalore, India), 0.5 mM dNTPs, 2.0 mM $MgCl_2$,

Table 5: Sequences of primers used for DNA profiling of CIM-Unnati

S. No.	Primer name	Sequence 5' to 3
1	UBC 813	CTCTCTCTCTCTCTCTT
2	UBC 816	CACACACACACACACAT
3	UBC 818	CACACACACACACACAG
4	UBC 822	TCTCTCTCTCTCTCTCA
5	UBC 823	TCTCTCTCTCTCTCTCC
6	UBC 824	TCTCTCTCTCTCTCTCG
7	UBC 825	ACACACACACACACACT
8	UBC 828	TGTGTGTGTGTGTGTGA
9	UBC 829	TGTGTGTGTGTGTGTGC
10	UBC 830	TGTGTGTGTGTGTGTGG
11	UBC 836	AGAGAGAGAGAGAGAGYA
12	UBC 841	GAGAGAGAGAGAGAGAYC
13	UBC 842	GAGAGAGAGAGAGAGAYG
14	UBC 843	CTCTCTCTCTCTCTCTRA
15	UBC 844	CTCTCTCTCTCTCTCTRC
16	UBC 845	CTCTCTCTCTCTCTCTRG
17	UBC 846	CACACACACACACACART
18	UBC 847	CACACACACACACACARC
19	UBC 848	CACACACACACACACARG
20	UBC 849	GTGTGTGTGTGTGTGTYA
21	UBC 850	GTGTGTGTGTGTGTGTYC
22	UBC 853	TCTCTCTCTCTCTCTCRT
23	UBC 854	TCTCTCTCTCTCTCTCRG
24	UBC 855	ACACACACACACACACYT

Y = C/T; R = A/G

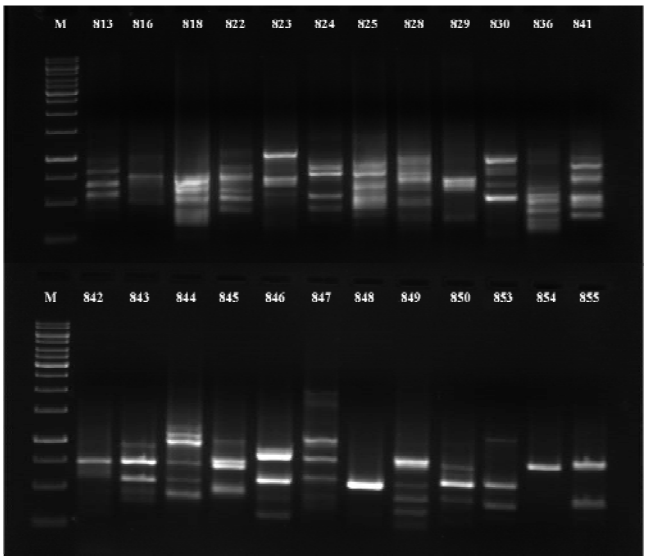

Figure 4: Characteristic PCR profile of MASP-19 (CIM-Unnati) with twenty four ISSR primers (UBC). M = DNA molecular weight marker (O'GeneRuler 1 kb DNA ladder fragments; 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250 bp)

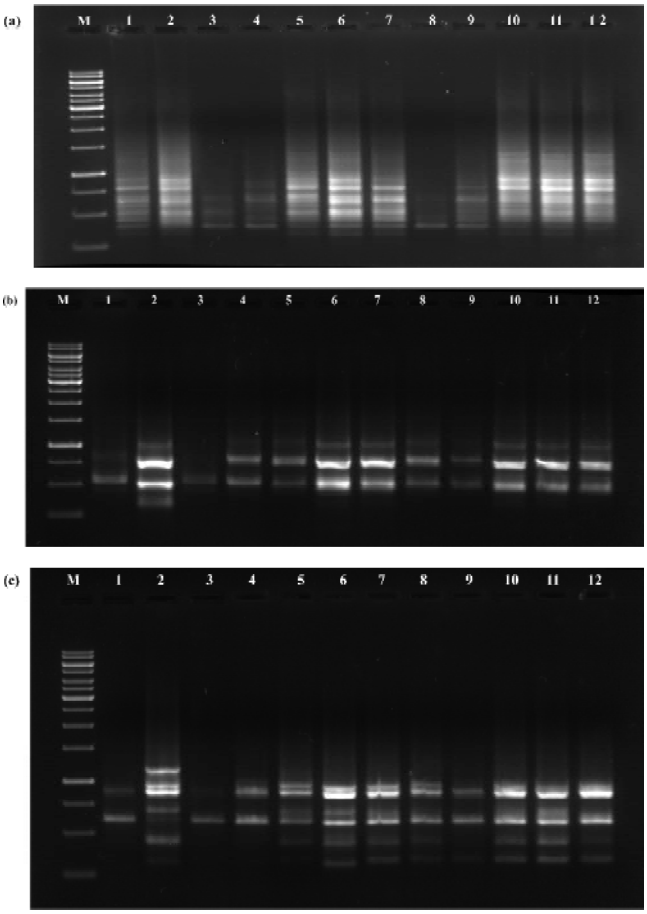

Figure 5: Comparative PCR profiles of MASP-19 (CIM-Unnati) and other menthol mint varieties with ISSR primers. (a) UBC 825; (b) UBC 845; (c) UBC 846. M = DNA molecular weight marker (O'GeneRuler 1 kb DNA ladder fragments; 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250 bp). Lanes 1-CIM-Unnati; 2-Gomti; 3-Kosi; 4- Kranti; 5-Saryu; 6-Kushal; 7-Kalka; 8-Sambhav; 9-Saksham; 10-MAS-1; 11-Damroo; 12-Himalaya

and 0.6 µM of each primer. For the amplifications, the thermal cycler was programmed as: 94˚C for 5 min; followed by 45 cycles of 94˚C for 1 min, X˚C (45˚C -58˚C, depending on the Tm of each primer) for 1 min, and 72˚C for 2 min; which was followed by final extension for 5 min at 72˚C. The amplification products were resolved on a 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide and their image was captured using a gel documentation system (Bio-Rad). A characteristic DNA profile (Fig. 4) was generated for MASP-19 (CIM-Unnati) using the selected twenty four ISSR primers (Table 5).

Comparative PCR profiles of MASP-19 (CIM-Unnati) and other menthol mint varieties with ISSR primers UBC 825, UBC 845 and UBC 846 are also shown (Fig. 5).

Table 6: Comparative essential oil composition of MASP-19 (CIM-Unnati), Kosi and CIM-Kranti

Composition	MASP-19	Kosi (Check)	CIM-Kranti (Check)
Limonene (%)	2.9	2.4	2.6
Menthone (%)	6.5	2.8	4.7
Isomenthone (%)	5.8	2.7	5.6
Menthol (%)	74.3	80.6	77.5
Neomenthol (%)	1.9	1.6	1.8
Pulegone (%)	0.4	0.3	0.4
Menthyl acetate (%)	2.7	2.4	2.0

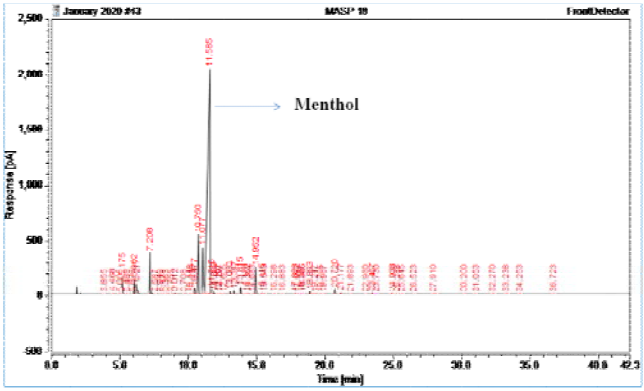

Figure 6: Essential oil profile of MASP-19 (CIM-Unnati)

Essential oil composition of CIM-Unnati: The essential oil composition of new variety in comparison to check variety Kosi and CV- Kranti is provided in Table 6; Fig.6. The physical attributes of the essential oil of CIM-Unnati are summarized in Table 7.

Table 7: Physical parameters of essential oil of MASP-19 (CIM-Unnati)

Physical parameter	Value
Refractive index (20°C)	1.4596
Optical rotation (20°C)	-37.24
Relative density (18.6°C)	0.9013
Solubility in 70% ethyl alcohol (18.6 °C)	2.3-2.4 volumes [a]

Recommended cultivation schedules for the variety CIM-Unnati

The new variety MASP-19 (CIM-Unnati) should be planted in the 2nd week of August for sucker production, whereas the planting time for essential oil production is 2nd to 4th week of February. Highest oil yield was obtained when a spacing of 60 cm between lines and 30 cm between plant to plant was followed. The recommended fertilizer application is NPK@120:80:60 kg/ha (1/3rd N, and full dose of P and K as basal, and remaining N in two equal split at 25-35 and 45-55 days after planting. The crop duration can be kept within 100-110 days after planting.

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NOTE

CIM-Unnati was released for commercial cultivation on the occasion of CSIR-CIMAP Kisan Mela on 31 January, 2020.

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