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NEW VARIETY RELEASE

CIM-Sudeeksha: A high herb-yielding and carvacrol-rich clone of Indian Oregano (*Origanum vulgare* L.)

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ABSTRACT

Oregano (Origanum vulgare L.) is a well-known spice with many applications in the flavoring business. Various liquor preparations, tomato sauces, condiments, baked meals like pizza, and salad dressings all contain dried Oregano leaves and essential oil. Due to no commercial cultivars or varieties, Oregano cultivation is currently not widespread in India. With a focus on improving herb production and essential oil yield, CSIR-CIMAP has developed a new genotype of Oregano OV-2, presently named CIM-Sudeeksha. During field evaluation testing, this new variety consistently outperformed herb and essential oil yield. This variety matures in 150 days after planting and has the potential to produce 65-68 q/ha of dry herb that can give an essential oil yield of 45-48 kg/ha. This cultivar has roughly 0.7% essential oil content on a dry weight basis, and the carvacrol content in the oil varies between 53.0-63.0%. The two main attributes of this cultivar are distinctive leaf morphology and carvacrol content in the essential oil. Additionally, this variety fulfills several DUS (Distinctness, Uniformity, and Stability) standards.

INTRODUCTION

Oregano (*Origanum vulgare* L.) belongs to the Lamiaceae family; it is a key medicinal and aromatic herb widely distributed throughout Europe, West Asia, and Central Asia (Verma *et al*., 2012a). In India, it is widely spread throughout the sub-temperate to temperate Himalayan regions and is commonly known as Himalayan marjoram or JungaliTulsi (Verma *et al*., 2010a). Oregano is a popular culinary herb with great economic significance due to its many traditional and modern uses. Traditional practitioners have used it to cure many ailments, such as digestive problems, menstrual irregularities, whooping coughs, and convulsive coughs (Oezbek *et al*., 2008). Oregano is now a common ingredient worldwide, especially for preparing meat, vegetables, and spices (Verma *et al*., 2012b). Its essential oil, primarily composed of carvacrol or thymol and many levels of terpene and p-cymene, has been employed in the cosmetic and pharmaceutical sectors and the food business (Chauhan *et al*., 2013). Carvacrol is a valuable raw ingredient in these sectors due to its known antibacterial, anticancer, fungicidal, and antioxidant properties (Verma *et al*., 2010b).

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Oregano agribusiness has not yet gain mainstream popularity in India due to a shortage of commercial varieties, even though Indian climate is conducive to Oregano production. Its domestication and agricultural development programs have received little attention thus far, which is the root cause. Currently, the most commercial Oregano imports are from other nations, including Turkey, Italy, the United States, Spain, and the United Kingdom, with just a limited amount of Oregano being harvested from wild sources without any focus on quality. Given the current status of Oregano production in India and its commercial relevance among culinary herbs in global trade, a superior plant type with high herb and essential oil yield was needed. As a result, a planned Oregano breeding programme was initiated at the CSIR-CIMAP Research Centre in Pantnagar, Uttarakhand, to develop a new variety of oregano.

MATERIALS AND METHODS

Under a genetic improvement program on important medicinal and aromatic plants, Oregano for high herb and oil yield was undertaken at CSIR-CIMAP, Research Centre, Pantnagar, Uttarakhand, in 2016. A population improvement method (Half-sib progeny selection) was applied by using 14 Oregano populations/germplasm (OGP-1 to OGP-14) collected from different geographical areas belonging to different altitudes of Uttarakhand Himalaya (Table 1). The collected accessions were allowed to acclimate to foot-hill conditions during 2016 and 2017 and were multiplied vegetatively by stem cutting. In 2018, all these Oregano accessions (OGP-1 to OGP-14) were planted side-by-side and allowed to flower. Natural random mating was allowed during flowering, and seeds were collected separately from each Oregano accession after maturity. Seed germination was observed only in seeds collected from accession OGP-7 and OGP-12 (Fig. 2).

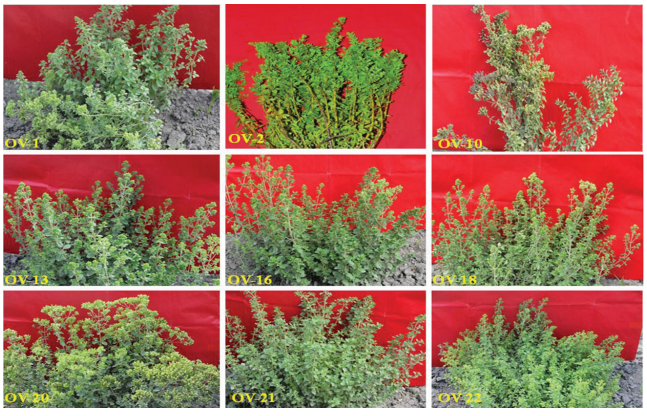

Figure 1: Newly developed morphologically distinct half-sib seed progenies of Oregano

A total of 22 half-sib seed progenies were developed using seeds collected from OGP-7 and OGP-12. All half-sib seed progenies were morphologically distinct (Fig. 1). Therefore, we intentionally selected all 22 half-sibs for further study. The vegetatively-multiplied half-sib progenies (OV-1 to OV-22) were first evaluated in an Initial Evaluation Trial (IET) (Plot size = 9m^2; Design= RBD; Replications= 3; row-to-row spacing 60 cm; plant-to-plant spacing of 30 cm) for their agronomic performance (Table 2).

Table 1: List of Oregano accessions, accession name, place of collection, and altitude.

S.No.	Accessions	Places of collection	Altitude (m)
1	OGP-1	Purara, Uttarakhand, India	1250
2	OGP-2	Purara, Uttarakhand, India	1250
3	OGP-3	Purara, Uttarakhand, India	1250
4	OGP-4	Nandaprayag, Uttarakhand, India	914
5	OGP-5	Gwaldham, Uttarakhand, India	1819
6	OGP-6	Kapkote, Uttarakhand, India	1,150
7	OGP-7	Gopeshwar, Uttarakhand, India	1,550
8	OGP-8	Tharali, Uttarakhand, India	1422
9	OGP-9	Mandal, Uttarakhand, India	1,700
10	OGP-10	Kausani, Uttarakhand, India	1,890
11	OGP-11	Chopta, Uttarakhand, India	2,608
12	OGP-12	Gwaldham, Uttarakhand, India	1,940
13	OGP-13	Karnaprayag, Uttarakhand, India	1,451
14	OGP-14	Purara, Uttarakhand, India	1250

RESULTS AND DISCUSSION

Based on the results of the IET, out of twenty-two vegetatively propagated half-sib progenies,

Table 2: Mean performance of selected clones of Oregano accessions in IET (Entries=14, Field design= RBD, Reps.= 3, Plot size= 9m²)

S. No.	Accession name	Plant height (cm)	Canopy width (cm)	Stem diameter (mm)	Branches per plant	L:S ratio	Internode length (cm)	Leaf petiole length (cm)	Leaf length (cm)	Leaf width (cm)	Fresh herb yield/ plot (kg)	Dry herb yield / plot (kg)	Oil percent (%)	Oil yield/ plot(g)
1	OV-1	47.73	35.12	1.75	19.13	1.02	3.77	0.81	1.11	0.71	10.10	3.70	0.30	30.07
2	**OV-2**	**50.67**	**42.16**	**2.34**	**52.27**	**1.40**	**0.90**	**1.04**	**1.84**	**2.31**	**18.37**	**8.12**	**0.30**	**83.11**
3	OV-3	32.63	28.20	1.16	37.53	1.30	2.23	0.81	1.83	1.52	5.33	2.65	0.16	7.93
4	OV-4	42.33	36.28	2.13	37.30	1.02	2.08	1.85	1.24	0.96	12.96	3.81	0.11	14.25
5	OV-5	35.00	28.35	2.02	39.23	0.82	2.02	0.82	1.15	0.91	12.34	4.41	0.16	18.41
6	OV-6	28.87	18.25	1.51	36.31	0.80	2.40	1.22	1.23	1.12	3.24	1.22	0.16	5.18
7	OV-7	37.67	31.43	1.87	42.40	0.91	2.25	1.02	1.27	1.25	9.30	3.11	0.15	12.95
8	OV-8	34.40	28.25	1.76	24.23	1.31	2.21	1.26	1.21	1.30	2.91	0.82	0.17	2.92
9	OV-9	35.20	27.34	1.65	22.60	1.21	2.30	1.24	1.24	1.13	5.56	2.28	0.16	8.87
10	OV-10	46.90	32.25	2.14	22.30	0.83	2.72	1.27	1.16	0.97	3.35	1.02	0.15	5.04
11	OV-11	41.24	27.33	1.85	39.10	1.51	2.12	1.13	1.45	1.00	3.64	1.24	0.14	5.48
12	OV-12	37.74	32.34	2.16	32.30	0.90	2.42	1.34	1.22	0.86	4.56	1.82	0.16	7.28
13	OV-13	34.53	26.43	1.86	36.23	0.81	2.30	1.32	1.22	1.11	4.27	2.11	0.15	6.41
14	OV-14	33.67	32.23	2.16	34.11	1.31	2.08	1.04	1.27	0.85	4.82	1.33	0.16	7.63
15	OV-15	33.00	23.25	2.22	37.53	1.27	1.88	0.82	1.33	0.81	5.26	2.21	0.15	7.89
16	OV-16	29.85	24.16	1.86	36.50	1.29	1.75	1.13	1.22	0.81	5.42	2.24	0.13	7.05
17	OV-17	40.67	38.16	2.10	42.33	1.22	1.52	0.96	1.23	0.81	9.91	3.51	0.15	14.85
18	OV-18	32.53	24.55	1.85	37.60	1.27	1.42	0.88	1.25	0.91	6.22	2.85	0.17	10.56
19	OV-19	46.00	25.30	2.13	42.20	1.25	2.51	0.98	1.25	0.85	9.82	3.21	0.10	9.82
20	OV-20	34.57	33.21	2.13	37.07	0.92	2.15	0.92	1.34	0.85	4.84	1.41	0.22	10.19
21	OV-21	33.59	28.27	2.15	28.43	1.20	1.82	1.22	1.33	0.88	4.12	2.20	0.15	6.18
22	OV-22	24.90	33.21	2.15	34.22	1.22	2.10	1.13	1.25	0.92	4.64	1.24	0.12	5.13
	Range	**24.90-50.67**	**18.25-42.16**	**1.16-2.34**	**19.13-52.27**	**0.80-1.51**	**0.90-3.77**	**0.81-1.85**	**1.11-1.84**	**0.71-2.31**	**2.91-18.37**	**0.82-8.12**	**0.10-0.30**	**2.92-83.11**
	C.D. @5%	**0.524**	**0.248**	**0.032**	**0.303**	**0.024**	**0.136**	**0.047**	**0.052**	**0.041**	**0.203**	**0.049**	**0.010**	**0.06**
	SE(m)	**0.183**	**0.087**	**0.011**	**0.106**	**0.008**	**0.048**	**0.016**	**0.018**	**0.014**	**0.071**	**0.017**	**0.004**	**0.021**
	SE(d)	**0.259**	**0.123**	**0.016**	**0.149**	**0.012**	**0.067**	**0.023**	**0.026**	**0.020**	**0.100**	**0.024**	**0.005**	**0.029**
	C.V.	**0.853**	**0.504**	**1.0000**	**0.522**	**1.276**	**3.857**	**2.563**	**2.412**	**2.406**	**1.787**	**1.151**	**3.810**	**1.277**

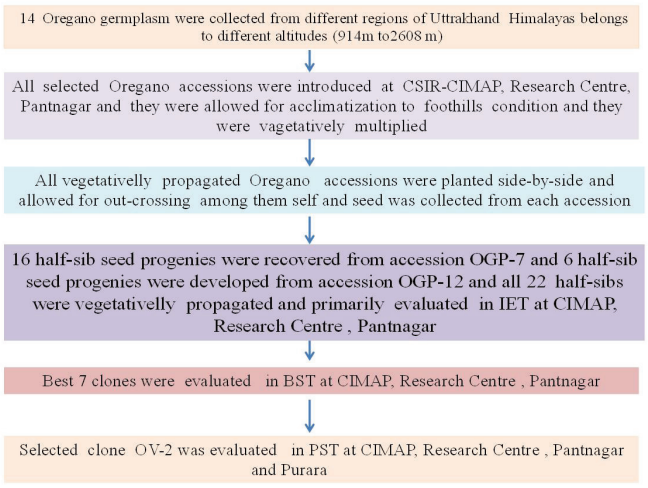

Figure 2: Breeding history of newly developed Oregano variety CIM-Sudeeksha (OV-2)

seven superior clones (OV-1, OV-2, OV-4, OV-5, OV-7, OV-17, and OV-19) were selected based on their herb and essential oil yield. In an Advance Variety Trial (AVT), these seven clones were evaluated for their essential oils, agronomic traits and chemical composition (Plot size=9m²; Design=RBD; Replications=3; row-to-row spacing 60 cm; plant-to-plant spacing 30cm). Results are summarized in Table 3. In AVT, the best-performing clone OV-2 was further selected based on herb yield and essential oil yield for evaluation in a Pilot Scale Trial (PST) (Plot size 800 m²). Throughout the study, the clone OV-2 maintained superiority over other accessions for herb yield and essential oil yield (Table 2-5). This high-yielding clone OV-2, christened as CIM-Sudeeksha, (Fig.3), is now proposed to be registered

Table 3: Pooled mean data on the performance of selected clones of oregano in AVT (Entries=7, Field design = RBD, Reps. = 3, Plot size = 9m²)

S. No.	Accession name	Plant height (cm)	Canopy width (cm)	Stem diameter (mm)	Branches per plant	L:S ratio	Internode length (cm)	Leaf petiole length (cm)	Leaf length (cm)	Leaf width (cm)	Fresh herb yield/ plot (kg)	Dry herb yield / plot (kg)	Oil percent (%)	Oil yield/ plot (g)
1	OV-1	52.17	37.32	1.74	19.25	1.02	4.10	0.90	1.12	0.72	10.24	3.76	0.31	30.34
2	**OV-2**	**54.17**	**45.31**	**2.48**	**53.62**	**1.42**	**1.03**	**1.15**	**1.87**	**2.31**	**18.70**	**8.14**	**0.34**	**83.37**
3	OV-4	43.60	35.56	2.08	38.68	1.03	2.08	1.85	1.23	0.96	12.52	3.82	0.12	14.34
4	OV-5	38.46	31.87	2.08	40.14	0.81	2.03	0.85	1.14	0.96	12.63	4.44	0.15	18.46
5	OV-7	38.40	33.52	1.88	43.95	0.89	2.25	1.04	1.26	1.25	8.25	3.12	0.15	12.86
6	OV-17	42.32	39.13	1.23	42.50	1.21	1.51	0.98	1.23	0.77	9.55	3.52	0.16	14.85
7	OV-19	46.41	26.32	2.28	42.26	1.25	2.51	0.98	1.24	0.86	9.90	3.22	0.12	9.79
Range		**38.40-54.17**	**26.32-45.31**	**1.23-2.48**	**19.25-53.62**	**0.81-1.42**	**1.03-4.10**	**0.85-1.85**	**1.12-1.87**	**0.72-2.31**	**8.25-18.70**	**3.12-8.14**	**0.12-0.34**	**9.79-83.37**
C.D. @5%		**0.447**	**1.926**	**0.926**	**0.561**	**0.023**	**0.075**	**0.016**	**0.017**	**0.015**	**0.072**	**0.037**	**0.019**	**0.542**
SE(m)		**0.144**	**0.297**	**0.297**	**0.180**	**0.007**	**0.024**	**0.005**	**0.006**	**0.005**	**0.023**	**0.012**	**0.006**	**0.174**
SE(d)		**0.203**	**0.420**	**0.420**	**0.255**	**0.010**	**0.034**	**0.007**	**0.008**	**0.007**	**0.033**	**0.017**	**0.009**	**0.246**
C.V.		**0.552**	**1.421**	**1.421**	**0.779**	**1.164**	**1.972**	**0.770**	**0.694**	**0.765**	**0.345**	**0.478**	**5.460**	**1.147**

Table 4: Mean performances of selected genotype/ clone (OV-2) of Oregano in the pilot-scale trial (PST) at CIMAP, Research Centre, Pantnagar (Plot size = 800 m²)

Attributes	OV-2
Plant height (cm)	57.21
Canopy width (cm)	47.34
Stem diameter(mm)	2.38
Branches per plant	62.14
L:S ratio	1.43
Internode length(cm)	1.05
Leaf petiole length (cm)	1.18
Leaf length (cm)	1.92
Leaf width (cm)	2.38
Fresh herb yield/plot (q) (800 m²)	10.1
Estimated fresh herb yield /ha (q)	126.33
Dry herb yield /plot (q) (800 m²)	5.23
Estimated dry herb yield /ha (q)	65.42
Oil percent (%) (Fresh weight basis)	0.35
Oil percent (%) (Dry weight basis)	0.7
Oil yield/plot (kg) (800 m²) (Fresh weight basis)	3.53
Estimated oil yield/ha (kg)(Fresh weight basis)	44.21
Estimated oil yield/ha (kg) (Dry weight basis)	45.79

Table 5: Mean performances of selected genotype/ clone (OV-2) of Oregano in pilot scale trial (PST) at CIMAP, Research Centre, Purara (Plot size = 800 m²)

Attributes	OV-2
Plant height (cm)	65.24
Canopy width (cm)	58.21
Stem diameter(mm)	2.67
Branches per plant	68.24
L:S ratio	1.52
Internode length(cm)	0.9
Leaf petiole length (cm)	1.31
Leaf length (cm)	1.95
Leaf width (cm)	3.21
Fresh herb yield/plot (q) (800 m²)	10.27
Estimated Fresh herb yield /ha (q)	128.46
Dry herb yield /plot (q) (800 m²)	5.39
Estimated Dry herb yield /ha (q)	67.48
Oil percent (%) (Fresh weight basis)	0.39
Oil percent (%) (Dry weight basis)	0.72
Oil yield/plot (kg) (800 m²) (Fresh weight basis)	4.005
Estimated Oil yield/ha (kg)(Fresh weight basis)	50.09
Estimated Oil yield/ha (kg) (Dry weight basis)	48.58

for its release as an improved cultivar for commercial cultivation.

STATEMENT OF DISTINCTION/BREEDER'S CLAIM

The variety CIM-Sudeeksha is an erect plant type with dark green leathery leaves having an acute, undulated margin and broad ovate shape. Its symmetric growth habit results in a round plant canopy. The stem is light green, and the DUS characteristic attributes of the new Oregano variety are summarized in Table 6.

DNA profile of the new Oregano variety CIM-Sudeeksha (OV-2)

The DNA isolation was carried out from the plant leaves using the modified cetyltrimethylammonium bromide (CTAB) method(Doyle and Doyle, 1987). The quantification of DNA was done using a Nanodrop ND- 2000 spectrophotometer. Polymerase chain reactions (PCRs) for DNA profiling were performed in 10µl. Each reaction contained 25ng DNA, 1X PCR reaction buffer, 1.5 U Taq DNA polymerase (Genei, Banglore, India), 0.5 mM dNTPs, two mM Mgcl2, and 0.2 µM of each primer. For amplification, the thermal cycler was programmed at 94 °C for 4 min, with 35 PCR cycles at 94 °C for 1 min, X °C (depending on the Tm of each primer) for 1 min, and 72 °C for 90 sec; which was followed by final extension for 7 mins 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 (G: Box, Syngene Frederick, USA). Comparative PCR profiles of OV-2 and other half-sib seed progenies of Oregano with ISSR markers are shown in figure 4.

Table 6: DUS characters of newly identified Oregano strain

Attributes	OV-2
Growth habit	Bushy
Plant height (cm)	57.0-64.0
Canopy width (cm)	47.0-51.0
Stem	Circular, hard, light green
L:S ratio	1.43
Internode length(cm)	1.05
Leaf petiole length (cm)	1.18
Leaf length (cm)	1.92
Leaf width (cm)	2.38
Leaf colour	Dark green
Leaf arrangement	Opposite
Leaf texture	Thick and rigid
Leaf shape	Ovate
Adaxial leaf surface	Moderately hairy
Abaxial leaf surface	Hairy
Odour	Herbaceous, Spicy
Carvacrol content (%)	53.05-63.28%

Figure 3: General view of morphologically distinct selected Oregano clone (OV-2)

Essential oil composition of the variety, CIM-Sudeeksha

The physical attributes of the essential oil of Variety CIM-Sudeeksha are summarized in Table 7. The essential oil composition of new Oregano variety is provided in Table 8, Fig.5.

Table 7: Physical parameters of essential oil of OV-2 (CIM-Sudeeksha)

Physical parameters	Value
Appearance	Clear, mobile liquid
Color	Yellow
Optical rotation (20.0 °C)	-0.306°
Refractive index (20.0 °C)	1.5002
Relative Density (29.6 °C)	0.9034
Miscibility in 80% ethanol (27.5 °C)	1.5 Volume

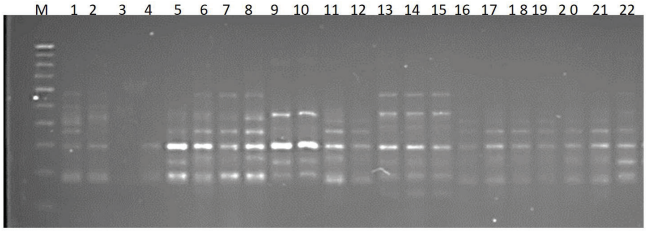

Figure 4: Comparative PCR profile of OV-2 (S. No. 2) (CIM-Sudeeksha) and other half-sib seed progenies of oregano with ISSR marker.

Table 8: The essential oil composition of OV-2 (CIM-Sudeeksha) at pre-flowering stage

Composition	OV-2
α-Thujene %	0.3-1.4
α-Pinene %	0.81-0.96
Camphene%	0.32
1-Octen-3-ol %	0.45
3-Octanone%	0.23
Myrcene %	2.24-2.53
δ−3−Carene %	0.09
α-Terpinene %	2.49-3.11
p-Cymene %	8.01- 8.29
β-Terpinene %	24.1-23.0
cis-Sabinene hydrate %	0.11
Linalool %	0.05-0.06
trans-Sabinene hydrate %	0.19
Borneol %	0.68
Terpinen-4-ol %	0.33-0.38
Thymol %	0.59-0.68
Carvacrol %	53.05-63.28
Carvacrol acetate %	0.30-1.24
β-Caryophyllene %	0.39-0.62
β-Bisabolene %	0.2
Spathulenol %	0.26
Caryophyllene oxide %	0.14

Recommended cultivation schedules for the variety CIM-Sudeeksha

Under foothill conditions, the new variety OV-2 (CIM-Sudeeksha) should be planted during 1st fortnight of October for herb and essential oil production. The highest oil yield was obtained when the spacing of 60 cm between lines and 30 cm between plants was made. The recommended time for the first harvest is in the 1stweek of March and subsequent in June. Under mid-hill conditions, the optimum planting time for herb and essential oil production is 2nd fortnight of February. The highest oil yield was obtained when the spacing of the 40 cm between lines and 30 cm between plant-to-plant was followed. The recommended duration for the first harvest is in the 1st week of May and subsequent July. The crop duration is 150-210 days after planting under both foothill and mid-hill conditions.

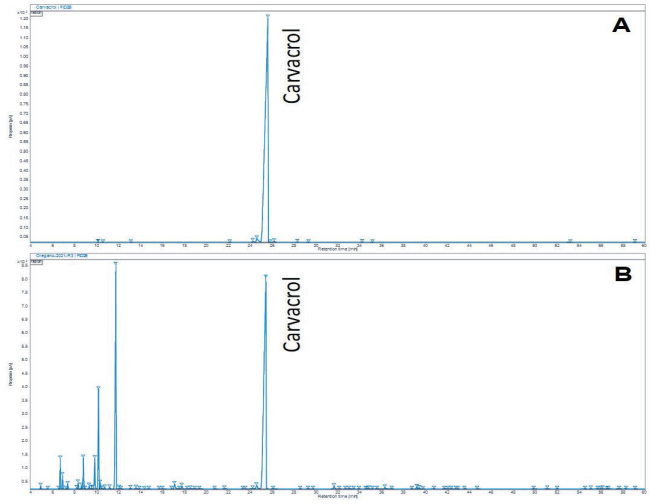

Figure 5: Chromatographic profile (GC-FID) of the essential oil of *Origanum vulgare* variety CIM-Sudeeksha (A: standard; B: essential oil).

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NOTE

CIM-Sudeeksha was released for commercial cultivation on the CSIR-CIMAP Annual day celebration on 22nd August 2022.

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