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Standardization of agro-techniques on the growth, yield and chemical composition of ginger lily (*Hedychium spicatum* Smith) in western Himalaya

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ABSTRACT

Hedychium spicatum, a genus known as ginger lilies, distributed in sub-tropical to sub-temperate zones of south and south-east Asia, and Madagascar regions. Its availability is 1000 to 2800 m altitudes. Two trials following, RBD was laid out in open and under tree shades of Kinnow, Morus alba and Grevillea robusta. Another trial was laid out using Melia azedarach, Jacaranda acutifolia and Morus alba as shade trees to study the growth and yield of H. spicatum in different years. The rhizomes were used for the planting. First trial was planted in January, 2003 and second in January, 2005, first harvested in January, 2006 and in 2008. Growth parameters was recorded. FYM 15t ha^{-1} showed better results in 1st trial. . However, in second experiment the rhizome yield under Melia azedarach was statistically significant. However, Jacaranda produced the highest significant yield of rhizome. The flower length was maximum in Kinnow shade and minimum in open conditions. The spacing had no significant effect on growth parameters. The aim of this study was to assess the variation in oil contents of cultivated Hedychium spicatum at lower altitude (1325 m msl), under growing different tree species, collected from three locations at CSIR-IHBT, Palampur farm. The yield of rhizomes in 2nd and 3rd year's harvest were statistically significant under the shade. In both the experiments, Kinnow shade was statistically significant and produced 16t ha^{-1} yield of rhizome after three years. In 25x25 cm spacing producing statistically significant rhizome yield in 2nd and 3rd years subsequently in first trial GC-MS (Gas chromatography-mass Spectrometry) analysis revealed the presence of 28 volatile components with the major constituents including 1,8-Cineole (60.7-65.7), δ-Cadinene (1.4-2.8%), Elemol (2.4-3.6%), α-Cadinol (2.5-3.3%) and Eudesmol (7-epi-alpha) (8.5-11.3%). The H. spicatum with high concentration of 1,8-Cineole may be recommended for treatment of COPD (chronic obstructive pulmonary disease) patients and reduced severity of dyspnea.

***Keywords*:** Agrotechniques, essential oil, *Hedychium spicatum*, rhizome, yield.

It belongs to family Zingiberaceae (Kapoor Kachari). A perennial rhizomatous herb *H. spicatum* is distributed in Western and North-Eastern Himalayas ranging from 1000 to 2800 m altitude. The rhizome of *H. spicatum* is used for curing several diseases as these rhizomes are stomachic, carminative, stimulant and tonic, and are used in dyspepsia in the form of powder or decoction. It is used in general anasarca, bad taste, colic, enteric fever, sore throat, asthma and bronchitis Kurup *et al*., 1979), eye disorder, liver complaints, urinary disorders, gastric disorder, vomiting, body ache, cuts and wounds, rheumatism and inflammation. They are ingredients of perfumed baits for fish cosmetic powders used for promoting hair growth. The powdered form called " abir" is also used tobacco Paan. In Almora the tribes used it fresh root (10g) in asthma and internal injury (Arya and Parkash, 2000). It is used in Ayurvedic preparations like Shyatyadichurna, Shatyadei Quath, Himanshu Tail, Sateyadivarga,Chandraprabha-vati and Agastyaharitaki rasayana. In the Unani system of medicine, it is considered as aphrodisiac. Rhizome is also used in snakebite. The essential oil recovery by solvent extraction is 2.3 to 4.0 per cent. The major constituents are 1,8–cineole (50.13%), α-cadinol (6.48%) δ-cadinene (2.73%), epi- α – eudesmol (1.47%) and α –terpinolene (1.26%).

ts biological activities including anthelmintic, fungicidal antioxidant, cytotoxic, antimicrobial, antifungal and anti-inflammatory (Prakash *et al.*, 2012., Koundal *et al.*, 2015, , Rajasekaran *et al.*, 2012). It can be integrated with agro-forestry/social forestry for the benefit of society, especially in hills of H.P. We had also released a cultivar of *H. spicatum*, "Him- Kachari".

MATERIALS AND METHODS

The field trial was conducted at Biodiversity Garden of CSIR-IHBT Institute of Himalayan Bioresource Technology, Palampur (1325 m above msl, 32^{0}06′05"N, 76^{0}34′10"E) situated in the mid hills of Himachal Pradesh, India. At the time of laying the experiment, soil samples from 0 to 15 and 15 to 30 cm depth were taken and analysed for physico-chemical properties. The soil was silty clay loam in texture and acidic in nature. The average of top and sub soil organic carbon was 2.25% in open conditions. While in *Morus* 1.90%, in *Grevillea* it was 2.92%. The available N P and K, was 197 kg ha^{-1}, 22.5 ppm , 459.5 ppm in open field. In *Morus* it was 175 kg ha^{-1}, 6.25 ppm and 180 ppm, and in *Grevillea*

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it was 192.5 kg ha^{-1}. 6.50 ppm and 110 ppm. In Kinnow, it was 210 kg ha^{-1}, 14.5 ppm and 170 ppm, while its organic matter content was 2.68 per cent.

The average rainfall during the period of study 2860.80 mm of which more than 80 per cent is received from July and August months. Average maximum and minimum temperatures were 24.90^{0}C and 10.88^{0}C, respectively. December to February was the coolest spell when maximum and minimum tɛ nperatures were 17.8^{0}C and 3.9^{0}C, respectively. Average relative humidity was 50.10 per cent during their crop season. In First experiment, three number spacing (25 x 25cm, 50 x 25cm and 50 x 50cm) and three plant species (*Kinnow, Morus and Grevillea*) as shade were laid. The tree plantation spacing was 5.0 x 5.0m and the bed size was 4.5 x 4.5m. In the first experiment FYM was not a main factor but here we have used tree species as a factor. In first experiment we have applied FYM 15t ha^{-1} at the time of bed preparation before planting as a basal dose. The field experiment (R & D) following completely randomized block design having, three plant species (*M. azedrach, J. acutifolia and M. alba*) as shade trees and two number spacing (50 x 50cm and 75 x 75cm), along with three number FYM application (15, 30 and 45 t ha^{-1}) Figure: 1a, 1b and 1c. The land was prepared very well manually. The rhizomes of uniform size about 5.0 - 6.0cm length and 3.0 to 4.0 cm width were used in both the experiments for plantation. Hardly two irrigations were given per year.

The cultivated fresh rhizomes of *H. spicatum* from different locations (*M. azedracht, J. acutifolia* and *M. alba*) were collected during the month of January, 2017 from Biodiversity garden under High Altitude Biology division, earlier it was Biodiversity Division, CSIR-IHBT, Palampur (H.P.), India. The plant specimens were characterized by the taxonomist of the CSIR-Institute of Himalayan Bioresource Technology, Palampur. A specimen of target plant was deposited in the herbarium of CSIR-IHBT (voucher # PLP 1109), Palampur, India.

Fresh rhizome of *H. spicatum* grown under *Melia* (2.0 kg), *Jacaranda* (2.0 kg) and *Morus* (2.1 kg) were cut into small pieces and hydrodistilled for 3 hours using Clevenger-type apparatus. The EOs obtained were pale yellow in color and yielded 0.12, 0.12 and 0.1% (v/w). EOs were dehydrated over anhydrous sodium sulfate and stored in sealed vials at 4°C until used for further detailed characterization.

The chemical composition of obtained EOs were analyzed by gas chromatography (GC) on Shimadzu GC 2010 equipped with ZB-5MS (J & W Scientific, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 ìm film thickness) and FID detector. The GC oven temperature program was as follows, 70°C (initial temperature) held for 4 minutes and then @ 4°C$minute^{-1}$ to 220°C and held for 5 minutes. Injector temperature, 240°C, detector temperature, 260°C, injection mode, split. Carrier gas was nitrogen at column flow rate of 1.05 mL$minute^{-1}$ (65.3 kPa). The gas chromatography$mass^{-1}$ spectrometry ($GCMS^{-1}$) analyses of the oils were performed using a Shimadzu QP 2010 equipped with AOC-5000 auto-injector using a ZB-5MS (J & W Scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm i.d., 0.25 ìm thickness). The GC oven temperature was 70°C for 4 minutes and then to 220°C at 4°C/minute and held for 5 minutes. Injector temperature, 240°C, interface teɾ ıperature, 250°C, acquisition mass range, 800–40 amu; ionization energy, 70 eV. Helium was used as carrier gas. EOs composition was analyzed in triplicate and the results presented as mean ± standard error using sigma stat 3.5 software as shown in table- 6.

RESULTS AND DISCUSSION

Growth and yield parameters

The observation was recorded after sprouting in the rhizomes in the month of May, 2003 and 2005. Various growth parameters such as, sprouting and growth period, no. of leaves $plant^{-1}$, no. of side tillers $plant^{-1}$, plant height and flower length, rhizome yield kg sqm^{-1} and total yield (t ha^{-1}) of two years and three years was recorded (Table 1,2, 3, 4, 5, 6, 7 and 8). In case of first experiment the maximum no. of leaves per plant was recorded in *Morus*, while number of side plant (tillers) was 3.1 no. in Kinnow shade (Table 1). The flower initiation were started in 1st week of July and fruiting upto the end of August. In case of Kinnow, it was observed first in about 10 days difference in sprouting from open and other under shade trials. The length of flower was also maximum in Kinnow shade about 30 to 40 cm in overall length, while in other shade species it was found to decrease respectively. The minimum size of flower was recorded in open trial. In this way, in *Morus* and *Grevillea* are the dense plantation and the difference of light intensity. The plant height was maximum under *Morus* shade. In the case of yield of rhizome, these parameters are statistically significant and significantly highest yield was recorded in the Kinnow shade 14.0 tha^{-1} and 16 t ha^{-1} in 2nd and 3rd years, respectively. However the rhizome yield of two and three years harvesting was statistically significant (Table 1 and 2). In terms of growth parameters, the flower size was maximum in 25 x 25 cm spacing and decreased toward as spacing increased (Table 2). Similar observations were recorded for other parameters such as no. of leaves, no. of tillers per plant and plant height. The lower spacing produced maximum no. and height while in wider spacing the trend was in decreasing order (Table 2).

Every shade was significant in terms of 2nd and 3rd years rhizome yield. There was no significant difference recorded in terms of growth parameters year wise. But the dose of FYM 30tha^{-1}, produce better plant height in comparison to with lower application of FYM, which was also produced better results in terms of growth

Table 1: Influence of shade trees on the growth parameters and yield of *H. spicatum*.

Shade trees	No. of leaves plant^{-1}	No. of tillers plant^{-1}	Flower length (cm)	Plant height (cm)	Rhizome weight at 2 years		Rhizome weight at 3 years	
					(Kg sqm^{-1})	(t ha^{-1})	(Kg sqm^{-1})	(t ha^{-1})
Kinnow	9.2	3.1	30.2	109.0	1.4	14.0	1.6	16.0
Morus	9.5	2.9	25.8	122.4	1.2	12.3	1.4	14.2
Grevillea	7.9	2.8	27.7	98.2	1.3	12.7	1.5	15.0
Open	8.9	2.4	24.0	108.4	1.2	12.0	1.4	14.0
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**0.036**	**0.362**	**0.038**	**0.380**

Table 2: Influence of plant spacing on the growth parameters and yield of *H. spicatum*.

Spacing (sq cm)	No. of Leaves plant^{-1}	No. of tillers plant^{-1}	Flower length (cm)	Plant height (cm)	Rhizome weight at 2 years		Rhizome weight at 3 years	
					(Kg sqm^{-1})	(tha^{-1})	(Kg sqm^{-1})	(tha^{-1})
25x25	9.2	2.9	29.1	119.5	1.5	15.3	1.7	17.4
50x25	8.9	2.8	27.0	105.9	1.3	13.3	1.5	14.8
50x50	8.5	2.8	24.7	103.1	1.0	9.7	1.2	12.3
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**0.031**	**0.314**	**0.033**	**0.329**

Table 3: Growth parameters of *H. spicatum* growing under *M. azedarach* shade trees with the application of FYM and spacing.

	***Melia azedarach* plantations, 2005**				***Melia azedarach* plantations, 2006**			
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**
F1	51.44	9.27	1	14.92	77.61	10.89	2.44	24.72
F2	54.78	9.78	1	12.53	77.33	10.72	2.22	23.00
F3	51.00	9.33	1	13.14	76.89	10.56	2.11	23.83
SEm (±)	**2.73**	**0.22**	**0**	**0.72**	**6.01**	**0.40**	**0.33**	**2.61**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Spacing								
S1	52.52	9.43	1	13.85	79.81	10.81	2.37	24.89
S2	52.30	9.49	1	13.20	74.74	10.63	2.15	22.81
SEm (±)	**2.23**	**0.18**	**0**	**0.59**	**4.91**	**0.33**	**0.27**	**2.13**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
FYM x Spacing								
F1X S1	49.22	9.30	1	15.06	81.33	10.89	2.11	25.89
F1X S2	53.67	9.23	1	14.78	73.89	10.89	2.78	23.56
F2X S1	55.44	9.67	1	11.56	76.22	10.89	2.67	22.67
F2X S2	54.11	9.89	1	13.50	78.44	10.56	1.78	23.33
F3X S1	52.89	9.33	1	14.94	81.89	10.67	2.33	26.11
F3X S2	49.11	9.33	1	11.33	71.89	10.44	1.89	21.56
SEm (±)	**3.87**	**0.31**	**0**	**1.02**	**8.50**	**0.57**	**0.47**	**3.68**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 4: Growth parameters of *H. spicatum* growing under *M. azedarach* shade trees with the application of FYM and spacing and rhizome yield.

	***Melia azedarach* plantations, 2007**				***Melia azedarach* plantations, 2008**				
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Yield (t/ha)**
F1	77.61	10.89	2.44	24.72	79.78	10.50	2.17	20.00	4.61
F2	77.33	10.72	2.22	23.00	76.83	10.72	3.00	18.00	4.36
F3	76.89	10.56	2.11	23.83	79.06	10.83	2.28	18.44	4.12
SEm (±)	**6.01**	**0.40**	**0.33**	**2.61**	**4.11**	**0.35**	**0.44**	**1.34**	**0.78**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Spacing									
S1	79.81	10.81	2.37	24.89	79.59	10.74	2.78	19.19	5.54
S2	74.74	10.63	2.15	22.81	77.52	10.63	2.19	18.44	3.18
SEm (±)	**4.91**	**0.33**	**0.27**	**2.13**	**3.36**	**0.28**	**0.36**	**1.10**	**0.64**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**2.02**
FYM X Spacing									
F1X S1	81.33	10.89	2.11	25.89	82.44	10.11	1.89	20.78	5.10
F1X S2	73.89	10.89	2.78	23.56	77.11	10.89	2.44	19.22	4.12
F2X S1	76.22	10.89	2.67	22.67	78.56	11.44	3.78	19.11	6.09
F2X S2	78.44	10.56	1.78	23.33	75.11	10.00	2.22	16.89	2.63
F3X S1	81.89	10.67	2.33	26.11	77.78	10.67	2.67	17.67	5.43
F3X S2	71.89	10.44	1.89	21.56	80.33	11.00	1.89	19.22	2.80
SEm (±)	**8.50**	**0.57**	**0.47**	**3.68**	**5.81**	**0.49**	**0.62**	**1.90**	**1.11**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 5. Growth parameters of *H. spicatum* growing under *J. acutifolia* shade trees with the application of FYM and spacing.

	***Jacaranda acutifolia* plantations, 2005**				***Jacaranda acutifolia* plantations, 2006**			
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**
F1	50.39	9.22	1	15.44	80.72	10.94	3.50	25.50
F2	46.94	9.28	1	13.06	78.11	10.78	3.50	25.89
F3	51.39	9.61	1	15.19	75.87	10.78	2.83	23.94
SEm (±)	**3.14**	**0.20**	**0**	**1.05**	**3.56**	**0.19**	**0.28**	**0.97**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Spacing								
S1	46.89	9.30	1	14.56	74.65	11.04	3.63	24.22
S2	52.26	9.44	1	14.57	81.81	10.63	2.93	26.00
SEm (±)	**2.56**	**0.16**	**0**	**0.86**	**2.91**	**0.16**	**0.23**	**0.79**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
FYM x Spacing								
F1X S1	44.33	49.22	1	14.33	77.44	11.78	3.44	23.67
F1X S2	56.44	53.67	1	16.56	84.00	10.11	3.56	27.33
F2X S1	43.22	55.44	1	13.22	80.67	10.67	4.11	26.11
F2X S2	50.67	54.11	1	12.89	75.56	10.89	2.89	25.67
F3X S1	53.11	52.89	1	16.11	65.84	10.67	3.33	22.89
F3X S2	49.67	49.11	1	14.28	85.89	10.89	2.33	25.00
SEm (±)	**4.44**	**3.87**	**0**	**1.49**	**5.03**	**0.27**	**0.40**	**1.38**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**0.86**	**NS**	**NS**

Table 6: Growth parameters of *H. spicatum* growing under *J. acutifolia* shade trees with the application of FYM and spacing and rhizome yield.

	***Jacaranda acutifolia* plantations, 2007**				***Jacaranda acutifolia* plantations, 2008**				
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Yield t/ha**
F1	80.72	10.94	3.50	25.50	73.56	10.94	2.78	21.94	8.40
F2	78.11	10.78	3.50	25.89	76.94	11.00	2.78	21.17	8.72
F3	75.87	10.78	2.83	23.94	75.78	10.89	2.22	21.56	8.23
SEm (±)	**3.56**	**0.19**	**0.28**	**0.97**	**4.40**	**0.27**	**0.28**	**2.04**	**1.00**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Spacing									
S1	74.65	11.04	3.63	24.22	76.63	11.07	2.70	21.93	9.60
S2	81.81	10.63	2.93	26.00	74.22	10.81	2.48	21.19	7.30
SEm (±)	**2.91**	**0.16**	**0.23**	**0.79**	**3.59**	**0.22**	**0.23**	**1.67**	**0.82**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
FYMxSpacing									
F1X S1	77.44	11.78	3.44	23.67	68.67	10.33	2.22	19.56	9.22
F1X S2	84.00	10.11	3.56	27.33	78.44	11.56	3.33	24.33	7.57
F2X S1	80.67	10.67	4.11	26.11	79.22	11.67	3.33	22.78	11.52
F2X S2	75.56	10.89	2.89	25.67	74.67	10.33	2.22	19.56	5.93
F3X S1	65.84	10.67	3.33	22.89	82.00	11.22	2.56	23.44	8.07
F3X S2	85.89	10.89	2.33	25.00	69.56	10.56	1.89	19.67	8.40
SEm (±)	**5.03**	**0.27**	**0.40**	**1.38**	**6.22**	**0.38**	**0.39**	**2.88**	**1.42**
LSD (0.05)	**NS**	**0.86**	**NS**	**NS**	**NS**	**1.19**	**1.24**	**NS**	**NS**

Table 7: Growth parameters of *H. spicatum* growing under *M. alba* shade trees with the application of FYM and spacing.

	***Morus alba* plantations, 2005**				***Morus alba* plantations, 2006**			
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**
F1	50.33	9.50	1	15.44	80.11	11.33	2.11	23.28
F2	50.89	9.56	1	15.89	85.33	11.17	1.78	24.22
F3	52.17	9.33	1	15.67	88.33	11.06	2.00	23.61
SEm (±)	**2.07**	**0.35**	**0**	**1.13**	**3.18**	**0.17**	**0.18**	**1.80**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Spacing								
S1	52.63	9.56	1	16.26	86.93	11.19	1.89	23.44
S2	49.63	9.37	1	15.07	82.26	11.19	2.04	23.96
SEm (±)	**1.69**	**0.28**	**0**	**0.92**	**2.59**	**0.14**	**0.15**	**1.47**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
FYM x Spacing								
F1X S1	50.56	9.67	1	15.89	83.11	11.33	1.78	23.56
F1X S2	50.11	9.33	1	15.00	77.11	11.33	2.44	23.00
F2X S1	52.33	9.67	1	16.67	93.22	11.11	2.11	24.67
F2X S2	49.44	9.44	1	15.11	77.44	11.22	1.44	23.78
F3X S1	55.00	9.33	1	16.22	84.44	11.11	1.78	22.11
F3X S2	49.33	9.33	1	15.11	92.22	11.00	2.22	25.11
SEm (±)	**2.93**	**0.49**	**0**	**1.59**	**4.49**	**0.24**	**0.25**	**2.55**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 8: Growth parameters of *H. spicatum* growing under *M. alba* shade trees with the application of FYM and spacing and rhizome yield.

	***Morus alba* plantations, 2007**				***Morus alba* plantations, 2008**				
FYM	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Plant height**	**No. of leaf**	**No. of tiller**	**Flower length**	**Yield t/ha**
F1	80.11	11.33	2.11	23.28	72.28	10.61	3.67	20.39	5.93
F2	85.33	11.17	1.78	24.22	74.06	10.83	3.33	19.56	5.68
F3	88.33	11.06	2.00	23.61	82.83	10.72	4.00	23.22	6.09
SEm (±)	**3.18**	**0.17**	**0.18**	**1.80**	**2.91**	**0.36**	**0.64**	**0.69**	**1.86**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**2.19**	**NS**
Spacing									
S1	86.93	11.19	1.89	23.44	76.15	10.67	4.00	22.19	5.65
S2	82.26	11.19	2.04	23.96	76.63	10.78	3.33	19.93	6.15
SEm (±)	**2.59**	**0.14**	**0.15**	**1.47**	**2.37**	**0.29**	**0.53**	**0.57**	**1.52**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**1.79**	**NS**
FYM x Spacing									
F1X S1	83.11	11.33	1.78	23.56	74.11	10.56	4.00	21.33	5.60
F1X S2	77.11	11.33	2.44	23.00	70.44	10.67	3.33	19.44	6.26
F2X S1	93.22	11.11	2.11	24.67	72.56	10.56	3.22	20.33	2.96
F2X S2	77.44	11.22	1.44	23.78	75.56	11.11	3.44	18.78	8.40
F3X S1	84.44	11.11	1.78	22.11	81.78	10.89	4.78	24.89	8.40
F3X S2	92.22	11.00	2.22	25.11	83.89	10.56	3.22	21.56	3.79
SEm (±)	**4.49**	**0.24**	**0.25**	**2.55**	**4.11**	**0.50**	**0.91**	**0.98**	**2.63**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

parameters. As the crop was harvested the statistically significant rhizome yield was recorded in lower dose of FYM after harvesting at three years (Table 6). In the case of Jacaranda some growth parameters, plant height, flower length, no. of leaves, etc., was also recorded better in lower dose of FYM (Table 5). In the case of interaction studies (FYM x spacing) no. of leaf was also statistically significant (Table 5 and 6). The dose of FYM in the case of *Jacaranda* shading the rhizome yield was highest in comparison to *Melia* and *Morus* (Table 6, 7and 8) Fig. 1d. In the case of spacing, the result was statistically significant in terms of yield of rhizome in 2nd and 3rd year (Table 2 and 4). It was statistically significant the rhizome yield. In first trial, in case of spacing, the growth parameters was statistically non significant.

Identification of components

The identification of EOs constituents were performed on the basis of their GC retention indices (RIs), determined for all volatile constituents using homologous series of *n*-alkanes (C_9-C_{24}) on ZB-5MS capillary column and using library search of National Institute of Standards and Technology (NIST) database (Stein, 1990), as well as comparing their RI and mass spectra with literature data (Adams, 2007).

Essential oil composition

The chemical compositions of essential oils extracted from the cultivated *H. spicatum* from three locations (fresh rhizome) at lower altitude were studied. The GC and GC-MS analyses revealed the presence of twenty-eight components in characterized sample (Table 9). The essential oil exhibited pale yellow color with characteristic fragrance. The essential oil was characterized by GC-MS and quantified by GC. The volatile components identified accounted for 91.6-94.4% of the essential oil. The volatile oil from rhizome of *H. spicatum* was dominated by oxygenated monoterpenes (62.9-68.5%) such as 1,8-cineole (60.7-65.7%), and α-terpineol (1.2-1.6%), oxygenated sesquiterpenes (16.1-21.6%) containing elemol (2.4-3.6%), t-muurolol (1.2-1.4%) and 7-epi-α-eudesmol (8.5-11.3%), while monoterpene and Sesquiterpene hydrocarbons present in less concentration (Table 9).

Previous literature reported rhizomes were contained highest 1,8-cineole (19.8-66.9%) exhibited as major volatile components (Koundal et al. 2015). We had also found that α-pinene, β-pinene, τ-muurolol, α-eudesmol, linalool, 4-terpineol and α-terpineol were in the similar concentration which is in agreement with previously published report.

Table 9: Chemical composition of the cultivated *H. spicatum* rhizome essential oil collected from the (CSIR-IHBT) farm from three different locations.

Compounds	[a]RI_{cal}	[b]RI_{lit}	Oil content (%) *M. Azedrach*	Oil content (%) *J. acutifolia*	Oil content (%) *M. alba*	Mode of identification
α-thujene	930	931	-	-	0.1 ± 0.00	KI, MS
α-Pinene	939	939	0.8 ± 0.01	0.7 ± 0.10	0.8 ± 0.01	KI, MS
Sabinene	977	976	0.1 ± 0.00	0.1 ± 0.01	0.2 ± 0.00	KI, MS
β-Pinene	983	980	1.6 ± 0.01	1.9 ± 0.47	1.7 ± 0.02	KI, MS
β-myrcene	991	991	0.2 ± 0.03	-	0.1 ± 0.00	KI, MS
p-Cymene	1030	1026	t	-	0.1 ± 0.00	KI, MS
Limonene	1035	1031	0.8 ± 0.02	-	0.8 ± 0.01	KI, MS
1,8-Cineole	1038	1033	65.3 ± 0.27	60.7 ± 1.91	65.7 ± 0.92	KI, MS
Linalool	1106	1098	0.5 ± 0.10	0.4 ± 0.10	0.4 ± 0.01	KI, MS
4-Terpineol	1187	1177	0.8 ± 0.01	0.6 ± 0.12	0.8 ± 0.01	KI, MS
α-Terpineol	1199	1189	1.6 ± 0.04	1.2 ± 0.07	1.6 ± 0.08	KI, MS
trans-Caryophyllene	1424	1418	0.2 ± 0.00	0.2 ± 0.01	0.2 ± 0.02	KI, MS
α-Humulene	1455	1454	0.3 ± 0.00	0.1 ± 0.02	0.1 ± 0.02	KI, MS
Aromadendrene (allo)	1465	1461	0.5 ± 0.20	-	-	KI, MS
γ -Muurolene	1479	1477	0.6 ± 0.05	0.2 ± 0.09	0.2 ± 0.06	KI, MS
Germacrene D	1485	1480	0.5 ± 0.02	-	0.1 ± 0.01	KI, MS
α-Muurolene	1501	1499	0.4 ± 0.01	0.2 ± 0.06	0.4 ± 0.10	KI, MS
γ-Cadinene	1517	1513	1.0 ± 0.01	1.1 ± 0.14	0.7 ± 0.01	KI, MS
δ-Cadinene	1522	1524	2.7 ± 0.02	2.8 ± 0.18	1.4 ± 0.01	KI, MS
Elemol	1556	1549	2.4 ± 0.02	3.5 ± 0.19	3.6 ± 0.09	KI, MS
Nerolidol E	1567	1564	0.4 ± 0.01	0.5 ± 0.02	0.4 ± 0.01	KI, MS
Germacrene D4 ol	1584	1574	-	0.1 ± 0.01	0.2 ± 0.01	KI, MS
Carryophyllene oxide	1588	1581	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.03	KI, MS
Humulene epoxide	1613	1606	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.03	KI, MS
γ-Eudesmol	1639	1630	0.4 ± 0.13	1.2 ± 0.03	0.9 ± 0.28	KI, MS
Compounds	**[a]RI_{cal}**	**[b]RI_{lit}**	**Oil content (%) *M. Azedrach***	**Oil content (%) *J. acutifolia***	**Oil content (%) *M. alba***	**Mode of identification**
t-Muurolol	1653	1645	1.4 ± 0.02	1.5 ± 0.07	1.2 ± 0.02	KI, MS
α -Cadinol	1666	1653	2.7 ± 0.05	3.3 ± 0.11	2.5 ± 0.09	KI, MS
Eudesmol (7-epi-alpha)	1671	1662	8.5 ± 0.18	11.3 ± 0.30	9.9 ± 0.54	KI, MS
Total [%]			94.0	91.6	94.4	
Monoterpene hydrocarbons				3.5	2.7	3.8
Oxygenated monoterpenes				68.2	62.9	68.5
Sesquiterpene hydrocarbons				6.2	4.4	3.1
Oxygenated sesquiterpenes				16.1	21.6	19.0

[a]*RI_{cal} Retention indices determined relative to n-alkanes (C_9-C_{24}) on the ZB-5 column.*
[b]*RI_{lit} Retention indices value of compounds in literature data (Adams., 2007).*

Regarding the standardization of agrotechniques of *H. spicatum*, the FYM application and spacing distance of both the experiment, even the interaction studies, the results recorded has produced a significant impact on final yield of rhizome after two and three years of plantation. Different plant spacing did not statistically significantly influenced the growth parameters of *H. spicatum,* like leaf, side tillers per plant, plant height and the flowers size etc. However, there was a definite trend in growth parameters**.** Singh et al. 2015, had studied on different spacing, and recorded different growth and yield parameters in the same crop. Our findings were statistically significant in the parallel spacing and fully agreed with the Singh et al. (2015), findings. In lower spacing 25x25 cm, the leaf number, number of side plants lets (tillers), flowers length per plant and plant height was recorded significant, while in wider spacing it was decreased simultaneously Gopichand *et al*. (2005). Various government agencies like National Medicinal Plant Board New Delhi, State Govt. H.P. and Deptt. Of

Figure-1. Cultivation of *H. spicatum* under different tree shades.

Fig. 1a. *H. spicatum* under *M. azedrach*

Fig. 1b. *H. spicatum* under *J. acutifolia*

Fig. 1c. *H. spicatum* under *M. alba*

Fig. 1d. Rhizomes of *H. spicatum*

Forest and environment, etc., are encouraging a policy in which intercropping cultivation of highly commercially important medicinal and aromatic crops have been must for the benefit of society. We have planted *H. spicatum* under the shade of trees. Government has promoted that all high valued and endangered medicinal plants should be raised as a crop under forest plantation or nearby forest by man made at the same habitat. The purpose of plantation in its natural habitat is of its sustainable conservation, especially of endangered plant species. *H. spicatum* is also an endangered medicinal plant in Western Himalaya and other parallel hilly states. It may become extinct very soon as indicated from the current rate of over exploitation of this species. The shade of three species was statistically significant in terms of crop yield, *i.e.*, rhizome yield in 2 and 3 years period. Similar result were observed by Rana *et al.* (2004), who has reported that *H. spicatum* can be raised as intercrop in the fruit orchard such as apple orchard. They further reported that the growth was exceptionally good, perhaps it provided congenial environment for its growth, i.e. partial shade, humidity and good mulch of apple leaves shed during winter. Thus there finding are absolutely agree with our findings. It was recorded during three years studies of some ever green deciduous tree species that maximum photoinhibation was recorded between 12.00 noon to 14.00 hrs. Even *Grevillea* showed decline 31% in $FvFm^{-1}$ ratio. Same Species showed 60% reduction in *Toona ciliata*, *Morus alba* and *Melia azedarach* Gopichand. (2014). It means a large portion of light was not utilized by tree plants or may disturbed their normal routine photosynthetic reaction with in leaves. Extra light was returned by tree species in atmosphere. It is evident that a sufficient amount of light was used by *H. spicatum* crop to perform their physiological activities as required by the plants. It means the partial shade of tree species play a very important positive role in running their routine physiological activities, resultant higher growth and yield was produced

Gopichand, (2014). These studies were recorded continuously in ten years. It was also recorded that some seasonal variations of the composition of its volatile oils of rhizomes of *H. spicatum* was also studied Koundal *et al.* (2015). The partial shade was also increased the growth and yield of *Ginkgo biloba*. Rana *et al.* (2004), has also reported that the propagation can be done by seeds and rhizomes. They further said that if the crop raised by seed, it will take time (3-4 years) for the formation of mature marketable rhizome. Therefore, it is always better to raise the crops from rhizomes. In the same pattern, we have also used the rhizomes for its plantation. Thus, their views are fully agreement with our findings.

In our field surveys, which we have been conducting in various regions of H.P. for our various R&D projects, we have also surveyed and studied its local and natural habitat. Its habitat was found as full of humus, highly fertile soil, high moisture and different types of forest trees for shade (broad leaf deciduous tree species), except in *Pinus* tree. Our field trials were similar habitat as our tree plantation now 13 years old, when we laid out this trial it was 10 years old. We observed a lot of humus, moisture, shade and fertile soil at our trial, which was result of fallen leaves in around the year and especially during dormant period. It may be assumed that these are congenial environment as soil moisture, humus and partial shed provided its basic requirement. Thus, we can recommend intercropping of *H. spicatum* in orchard, medicinal tree or other broad leaf forest trees. As per our experience, when shade is excess the plant should be planted at least 5.0m x 5.0m distance.

Our study revealed that very less qualitative and quantitative chemical variations were found in rhizome part of cultivated species of *H. spicatum* from different locations under tree species. Rhizome is the good source of 1,8-cineole and 7-epi-α-eudesmol. In previous literature reported studies on analysis of EOs constituents of *H. spicatum* rhizomes and roots displayed the presence of components, 1,8-cineole, β-pinene, limonene, α-pinene, myrcene, p-cymene, τ-muurolol, α-eudesmol, linalool, 4-terpineol, 10-epi-γ-eudesmol and α-terpineol as major volatile constituents Joshi *et al*., 2008). 1,8-Cineol mainly beneficial for human health to reduced severity of dyspnea and widely used in treatment of chronic obstructive pulmonary disease (COPD) patients Worth *et al.* (2009). In resultant, rhizomes essential oil composition of cultivated species of *H. spicatum* from different locations displayed very less qualitative and quantitative variation in chemical composition. It contain 1,8-cineole as its major constituents. Our result showed that high concentration of 1,8-cineole could be used as antibacterial, repellent, toxicant, grain protectant, food additive, drug formulation, perfumary and treatment of COPD patients. Our finding have great evidence for cultivated *H. spicatum* from three locations (CSIR-IHBT, Palampur) contain high concentration of 1,8-cineol as compared to previously reported literature in *H. spicatum* species that's why we can emphasized in all aspect that, this valuable medicinal plant can be cultivated in same natural conditions to enhance the productivity of secondary metabolite.

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