Article

Effect of growth hormones on seed germination and plant growth: with chemical components of *Hedychium spicatum Ham.ex.Smith.*

Gopichand[1], **R.L. Meena**[1], **A.K. Maurya**[2], **V. K. Agnihotri**[1], **R.D. Singh**[1]

[1]Department of High Altitude Biology, CSIR - Institute of Himalayan Bioresource Technology, Palampur, India

[2]Natural Product Chemistry and Process Development, CSIR - Institute of Himalayan Bioresource Technology, Palampur, India

E-mail: gopichand@ihbt.res.in

Received 29 June 2017; *Accepted 6 July 2017*; *Published 1 September 2017*

Abstract

Hedychium spicatum (Family: Zingiberaceae) is an aromatic crop, which is an endangered due to their high demand in pharmaceutical industries and unplanned, untimely harvesting by local population. For its conservation by its multiplication and cultivation, a seed germination trial (RBD) was laid out using selected growth hormones (IAA, IBA and NAA) with 100, 200 and 500 mg/l concentrations during various months of 2004-05. In the first year (July 2004), higher germination percentage (22%) was recorded for 500 mg/l IBA. While in 2005, in the months of April, May and June the seed germination percentage increased significantly subsequently in each treatment with different concentration. In 2005, in comparison to IAA and NAA, the statistically significant seed germination was recorded in the month of June (32.33%). The plant height and root length increased significantly in second year as compared to first year. In a separate study (2002 to 2004), significant increased growth was recorded in sprouting, plant height and number of leaves, fresh rhizome biomass and yield when treated with BAP 25 mg/l. The rhizomes were analyzed for their essential oils content and chemical composition by gas chromatography (GC-FID). Thirty-six components were identified, representing 89.1-95.9% of the total oil.

Keywords *Hedychium spicatum*; rhizome; hormones; essential oil; 1, 8-Cineole.

Ornamental and Medicinal Plants
ISSN 2522-3682
URL: http://www.iaees.org/publications/journals/omp/online-version.asp
RSS: http://www.iaees.org/publications/journals/omp/rss.xml
E-mail: omp@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Hedychium spicatum belongs to family Zingiberaceae. The word *Hedychium* originated from the Greek word '*hedys*' meaning sweet and '*chion*' meaning snow (Collet, 1921). In Hindi it is called Kapua-Kachari, Sanskrit Karpurakachali, Gandhashati and its trade name is Kapurkachari. Its English common name is Ginger lily, garland flower. The genus *Hedychium* consists of more than 46 species, which are found in different parts of the world. It is distributed in India, Bangladesh, Nepal, Pakistan, Malaysia, Japan and Europe. In India its 41 species are distributed in the Sub tropical Himalaya in the state of Assam, Arunachal Pradesh, Himachal Pradesh and Uttaranchal at an altitude of 1000-2800 m.

In the modern age, the world is attracting towards, “Herbals” in every sphere of life, especially in term of health concerns. The globe with scientific rationale and leads that are fast emerging to provide better health and life through plants and plant derived products (Stone, 2002). Herbal medicine is used by about 75-80% of the world population, especially in the developing countries, due to its better compatibility with the human body and lesser side effects (Kamboj, 2000; Zhang, 2017a, b). In all over world about 80% of the raw material used in various system of medicine, in the form of plants and are derived from the wild sources, mainly from the forests. In India, about 80% of medicinal and aromatic plants (MAPs) are collected from 17 million hectare of Indian forest (Chatterjee, 2002). In fact, the plant or its part is collected without paying any attention to the state of maturity, dried improperly and stored under unsuitable conditions. This type of unnatural collection from wild is unsustainable and is rapidly depleting the resource base and as a result many plant species are under threat of extinction. The *H. spicatum* is also an endangered plant species in Himachal Pradesh. Directorate of Indian system of Medicine & Homeopathic has included it in the list of 40 endangered medicinal plants on the verge of extinction.

In India, the fragrant rhizomes of *H. spicatum* are a considerable item for trade. The dried rhizomes are burnt as incense and a powdered form called "abir" is used for herbal ‘Holi’ colors. It is also used in perfuming tobacco that is chewed in pan. Floor mats are also made from the foliage due to its insecticidal properties. The oil extraction from its rhizome, which has a aroma (scent) somewhat like hyacinths, is so powerful that a single drop will render clothes highly perfumed for long time.

H. spicatum is also used for curing many diseases. The rhizome are carminative, emmenagogue, expectorant, stimulant, stomachic and tonic and is used in the general anasarca, bad taste in mouth, colic, fever, enteric fever and respiratory disorders, asthma and bronchitis (Prakash and Singh, 2001; Bhatt et al., 2009; Joshi and Tyagi, 2011), blood purification, eye disorder, gastric disorder, poultice for various aches and pains. Anti cancerous drug and indigenous medicines are prepared by using crude extract of the rhizome part of Hedychium species (Girri et al., 2010; Ghildiyal et al., 2012). Essential oils (EOs) obtained from this genus is extensively used in perfumery, in pharmaceutical industries as food preservative (Sabulal et al., 2007; Prakash et al., 2012). EOs has also demonstrated its potential biological activities including pediculicidal (Jadhav et al., 2007), antifungal (Rajasekaran et al., 2012), antimicrobial (Sabulal et al., 2007), antibacterial (Prakash et al., 2010), antioxidant (Koundal et al., 2015; Rawat et al., 2014; Joshi et al., 2008), anti-inflammatory (Lu et al., 20) and cytotoxic activities (Mishra et al., 2016).

It is used in Ayurvedic preparations like- Shatyadichurna, Shatyadei Quath and Himanshu Tail. In Ayurvedic medicinal texts, this herb has been described to be useful, among other things, in the treatment of swelling, asthma, fever and pain. It has been reported that in the remote tribal area of Almora district of Uttarakhand in western Himalaya, small pieces of its fresh root is partially cooked in burning flame and chewed with a glass of hot milk for the treatment of asthma and internal injury. The paste with hot water is given orally to the cattle and other domestic animals in case of stomach disorder. Sometime, it is also chewed by the local inhabitants to remove the bad smell from their teeth or mouth. The root paste along with wheat flour is given orally for the treatment of cough in cattle by Gaddi tribes in western Himalaya. The oil is highly resistant to temperature even at 120^0C and possesses insecticidal and fungicidal activity. The aqueous and ethanolic extract of *Hedychium spicatum* is having antitumour activities.

Our Institute (CSIR-IHBT) is also working on high valued important and endangered medicinal and aromatic crops. The *H. spicatum* is one of them and from more than last 10 years different types of R and D experiments are being done on it for its cultivation, domestication and sustainable conservation in this region and in its natural habitat (Koundal et al., 2015). *H. spicatum* is a shade loving plant species. We have more than 5-hectare land area under *H. spicatum* in various R and D experiment. In the present article we have used

some selected growth regulators on its seed germination, and its overall performance in the field, in terms of biomass and yield of rhizomes. Chemical characterization of rhizomes was also done, especially for essential oils. Some selected hormones were also used for the treatment of rhizomes.

2 Materials and Methods

The present study was conducted for a period of 4 years at the experimental farm of High Altitude Biology division of the Institute of Himalayan Bioresource Technology (CSIR), Palampur (H.P.), located at $32^0$06'05"N and $76^0$34'10"E situated in the mid hills of Himachal Pradesh, India at 1350m above msl. The experiment was laid out in February 2002. At the time of laying the experiment, soil samples from 0-15 and 15-30 cm depth were taken and analyzed for physico-chemical properties. The soil (typic Hapludalf) was silty clay loam in texture, normal to slightly acidic in soil reaction with high organic carbon percentage (Table 1). Available N, P and K were low, medium and high, respectively. On an average, cation exchange capacity was 116.65 µg per cm^2.

Table 1 Physico-chemical properties of the field soil of experimental site.

Property	Top soil (0-15 cm)	Sub soil (15-30 cm)
pH	6.3	5.8
Organic matter (%)	2.3	2.2
Carbon (%)	1.4	1.3
Exchangeable cation (µ g cm^{-2})	119.6	113.7
Available N kg ha^{-1}	198	196
Available P kg ha^{-1}	23	22
Available K kg ha^{-1}	538	381

The average annual rainfall during the period of study was 88.4 and 286.08 cm, respectively, of which more than 80% was received during June to September (source: CSK Himachal Pradesh Agricultural University, Palampur). Average maximum and minimum temperatures were 24.0°C and 10.90°C respectively, and the Sun shine hour was 345.80. December to February was the coolest spell when maximum temperature and minimum temperatures were 16.3°C and 4.6°C, respectively. Average relative humidity was 55% during both crop seasons.

2.1 Field experiment

The experiment for seed germination was laid out in July 2004, and again it was repeated in the month of April (1st week), 2005, having three no. of growth regulators Indole Acetic Acid (IAA), Indole Butyric Acid (IBA) and Nephthalene Acetic Acid (NAA) with three no. of concentrations 100, 200 and 500 mg/l., with water control. 200 no. of seeds were taken in each treatment and it replicate thrice in each case. The seeds dipping time was 15 minute of each treatment in both the years including water control. The soil culture medium was 1:1:1 i.e. equal ratio of sand, soil and FYM were used, in polyhouse in controlled conditions, temperature 25°C, ±2 and RH 65%. After 25 to 30 days, seeds were germinated. In the first year, especially in July, the germination started after 22 days, while in next year 2005, it was in 25 days. In the months of April, May and June the germination time was more than 30 days and continued up to 40 days. After August generally no germination was observed. Though some hardy seeds germinated but they died after 10-15 days of germination**.** Irrigation was given as or when it was required. After one month of seed germination, the plants were transferred to poly sleeves with same culture medium and stored inside the simple 50% agro-net shade.

The field experiment was conducted as per completely randomized block design having single plant spacing (50x50 cm) as first factor and three levels of PGR {GA3 (50, 100 and 250 mg/l), BAP(10, 25 and 50

mg/l) and IAA (10, 25 and 50 mg/l)} along with control as second factor, replicated thrice. The each plot size was 1.5x1.0 m^2. After primary tillage operations, well rotten FYM was applied as per the treatment and thoroughly mixed into the top soil. Rhizomes of uniform size of about 40 mm x 30 mm dip in solution for 30 minute, were planted on February 14, 2002. Irrigation was given as or when it required. Growth parameters, like sprouting, plant height, number of plantlets per plant, number of leaves per plant was recorded. Before onset of winter dormancy, when the leaves developed yellow color and started drying, the crop was uprooted from a each net area of plot size 1.5x1.0 m^2 in second year was the compound growth through 2 years.

2.2 Plant material (rhizomes) for distillation

The cultivated fresh rhizomes of *H. spicatum* were collected during the month of Jan 2017 while the roots were collected during the month of March 2017 from High Altitude Biology, CSIR-IHBT Palampur. 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.

2.3 Isolation of essential oils

Fresh rhizome of *H. spicatum*, 2.4 Kg and roots (500 gm) were chopped into small pieces and hydrodistilled for 3 hours using Clevenger-type apparatus. The EOs obtained was pale yellow in color, yield was found to be 0.22% and 0.06% (v/w). EOs were dehydrated over anhydrous sodium sulfate and stored in sealed vials at 4°C until used for further detailed analyses.

The extracted essential oil of *H. spicatum* (rhizome, 3 ml) was fractionated over silica gel (60-120 mesh) using column chromatography (60 g, 2.0 x 34 cm) with the solvent gradients of hexane: ethyl acetate (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 92:8 and 90:10 each 500 ml). On the basis of similar chromatograms on thin layer chromatography plates, fractions were pooled to afford 14 major fractions. The whole oil and fractions were evaluated with the help of ^{13}C nuclear magnetic resonance (NMR) (150 MHz) and proton NMR (600 MHz), run on Bruker Avance 600 MHz spectrometer (Bruker Bio Spin AG Industriestrasse, Fallanden, Switzerland) in deuterated chloroform solution.

2.4 Chemical analysis

2.4.1 GC/FID analysis

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 μm 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 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 (65.3 kPa).

2.4.2 GC/MS analysis

The gas chromatography/mass spectrometry (GC/MS) 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 μm 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 temperature, 250°C, acquisition mass range, 800–40 amu; ionization energy, 70 eV. Helium was used as carrier gas.

2.4.3 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 (Rana et al., 2004), as well as comparing their RI and mass spectra with literature data (Rajasekaran et al., 2012). The

major volatile components also confirmed by comparing the ^{13}C NMR spectra with the existing literature (Sabulal et al., 2007; Rawat et al., 2014).

2.5 Statistical analyses

EOs composition was performed in triplicate and the results presented as mean ± standard error using sigma stat3.5 software, as shown in Table 4.

3 Results

3.1 Seed germination

The seed germination was recorded after 22 days and it will continue up to 40 days after seed sowing. The higher percentage of seed germination was 22% in the treatment of IBA in 500 mg/l, in the same concentration the shoot length and root length was also recorded highest in comparison to all treatment (Table 2 and 3) The same experiment was also repeated in 2005, the seeds were started to germination after 30 days of sowing and continued up to 45 days. The parameter has been recorded in the month of April, May and June. All the parameters were statistically analysed. The germination percentage was lower in the month of April. As the climatic conditions improved simultaneously the germination percentage was increased in the month of May and up to first week of June. The higher concentrations produced promotory effect on seed germination in all the used growth regulators. The maximum germination was recorded in IBA in 500 mg/l, 16.33%, 24.33% and 32.33% in the month of April, May and June (Table 3) in 2005. In polyhouses conditions, in control the results were better in comparison to the lower concentrations of IAA in 2004, however, in 2005 it was lower percentage in comparison all the used growth regulators. Thus, on the basis of statistically analysis, it was observed that the parameters, as germination percentage, shoot length and root length was statistically non significant in 2004. It is clearly evident with the parameters as recorded in 2005 in the month of April, May and June all are statistically significant (Table 3). Lower concentrations of IAA, IBA and NAA producing lower seed germination other parameter in comparison to higher concentrations, all parameters were statistically analysed.

3.2 Field trial parameters

H. spicatum is a very important crop in terms of its medicinal use and commercial aspect. The crop cycle of it is only six months i.e. started from May and continue to October only. It is a deciduous nature of crop. It has started to sprouting on the returning of favourable climatic conditions, especially in the month of May and completed up to June end. In the month of late July and August, the flowering and fruiting were completed (Fig. 3 and 4). Due to heavy rains of Palampur region, the maturity of seeds is highly influenced by atmospheric adversities. In this way, the proper seed maturity is very less. All the growth parameters including sprouts no, tillering no, plant height and leaf no were also recorded (Fig. 1). The final harvesting has been done after two years completion of crop. The yield of rhizomes per treatment was also recorded (Fig. 1). It has been recorded that all the treatment as used in the trial , in the case of GA3 100 mg/l produced the highest rhizome yield 10.89 t/ha. In the case of BAP in 25 mg/l 20.39 t/ha rhizome yield was recorded (Fig. 1). However, in IAA 25 mg/l the maximum yield was obtained 11.72 t/ha. It was observed that lower and higher concentration of all hormons, as used in trial, produced lowest yield (Fig. 1). In the case of all the growth parameters as recorded in the experiment only the treatment of BAP 25 mg/l produced highest sprouting, total no of plantlets, no. of leaf per plant, plant height per plant and ultimately the highest yield of rhizome (Fig. 1).

Table 2 The effect of growth hormones on the seed germination, plant height and root length of *Hedychium spicatum* in 2004.

Growth Hormones	**07-07-2004**		
Treatments	**Germination (%)**	**Plant height (cm)**	**Root length (cm)**
IAA 100mg/l	4.00	0.92	2.80
IAA 200mg/l	16.00	2.46	3.02
IAA 500mg/l	19.50	4.12	3.30
IBA 100mg/l	7.00	1.77	2.93
IBA 200mg/l	14.50	2.06	3.42
IBA 500mg/l	22.00	2.48	3.48
NAA 100mg/l	5.00	0.60	3.20
NAA 200mg/l	17.00	2.06	3.36
NAA 500mg/l	21.00	3.98	3.26
Control	7.00	0.70	1.80
CD (P=0.05)	NS	NS	NS

Table 3 The effect of growth hormones on the seed germination, plant height and root length of *Hedychium spicatum* in 2005.

Growth Hormones	**12-04-2005**			**13.05.2005**			**16.06.2005**		
Treatments	**Germination (%)**	**Plant height (cm)**	**Root length (cm)**	**Germination (%)**	**Plant height (cm)**	**Root length (cm)**	**Germination (%)**	**Plant height (cm)**	**Root length (cm)**
IAA 100mg/l	10.00	5.40	2.07	20.33	9.50	2.20	20.00	8.58	2.55
IAA 200mg/l	14.00	6.10	2.33	22.00	10.30	2.60	23.67	10.26	2.97
IAA 500mg/l	17.67	7.07	2.63	26.33	11.10	2.97	28.00	11.69	3.19
IBA 100mg/l	11.00	5.03	2.53	16.67	9.67	2.53	26.33	9.07	2.92
IBA 200mg/l	13.67	5.93	2.80	19.67	11.37	2.87	28.33	11.13	3.26
IBA 500mg/l	16.33	6.90	2.97	24.33	11.97	3.20	32.33	12.73	3.50
NAA 100mg/l	12.67	5.57	2.07	15.67	9.03	2.33	20.00	8.48	2.69
NAA 200mg/l	15.33	6.10	2.37	19.33	11.53	2.60	22.67	10.94	2.96
NAA 500mg/l	20.33	6.50	2.60	24.00	14.03	3.03	25.33	13.07	3.53
Control	9.33	5.50	1.87	13.67	8.00	1.93	16.00	8.08	2.58
CD (P=0.05)	3.46	0.59	0.32	5.13	1.94	0.51	5.05	2.67	0.53

Consequently, due to immaturation of seeds, the germination percentage is very low. In the natural habitat, the seed germination is very rare. Therefore we have long programme of its cultivation, propagation and further multiplication in its own habitat, ultimately for its sustainable conservation. The seeds were collected from our High Altitude Biology (CSIR-IHBT, Palampur, H.P.), when the flower become red in the colour (Fig. 4).

In the case of plant height, also higher concentration of IAA, IBA and NAA produced higher plant height during both the years (Table 2). The root length's measurement was recorded at the transfer time, to polythene sleeves after 40 to 45 days of seed sowing. The result are almost parallel, there is no statistical significance in terms of root length. However, there was a trend in all the treatments towards lower to higher concentrations (Table 3). The root system in control was also good.

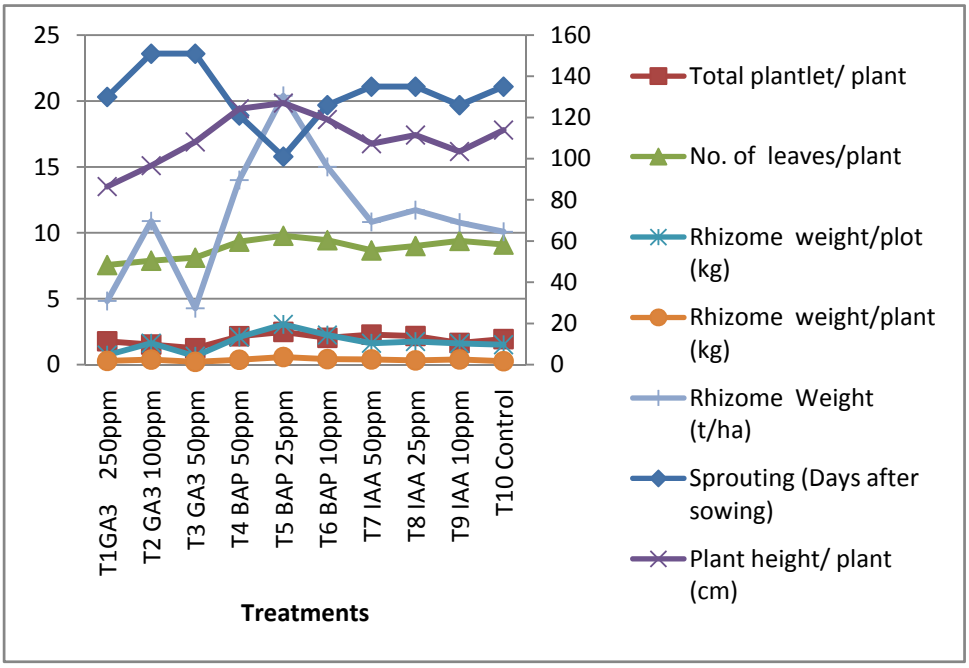

Fig. 1 Effect of selected growth hormons on different growth and yield parameters of *H. spicacum*.

Fig. 2 A nursery experiment.

Fig. 3 A field view of experiment.

Fig. 4 A mature stage of seed formation.

3.3 Essential oils composition

The chemical composition of essential oils extracted from the cultivated *H. spicatum* (fresh roots and rhizome) at lower altitude was studied (Table 4). The GC and GC-MS analyses revealed the presence of thirty-six components in analyzed sample (Table 4). The samples afforded essential oil with pale yellow color, characteristic fragrance. The essential oil was characterized by GC-MS and quantified by GC (Fig. 1 and 2). The volatile components identified accounted for 89.1-95.9% of the essential oil. The volatile oil from rhizome of *H. spicatum* was dominated by oxygenated monoterpenes (76.2%) ssuch as 1,8-cineole (73.3%), linalool (1.3%), 4-terpineol (1.1%) and α-terpineol (1.6%) while oxygenated sesquiterpenes (10.4%) containing elemol (1.0%), carryophyllene oxide (1.2%), t-muurolol (1.4%) and 7-epi-α-eudesmol (5.4%). The essential oil composition from root of *H. spicatum* was marked by the presence of monoterpenes (8.8%) represented α-pinene (1.4%), camphene (1.0%), β-pinene (3.7%) and limonene (1.4%) as major constituents. Oxygenated monoterpenes accounted (45.7%) of total oil with 1,8-cineole (42.8%), 4-terpineol (1.8%), while sesquiterpenes hydrocarbons (17.3%) and oxygenated sesquiterpenes (17.3%) containing E-β-farnesene(14.8%), γ-eudesmol (4.3%), bulnesol (9.5%) and elemol (1.4%) as major volatile components (Table 4).

Table 4 Chemical composition of the cultivated *Hedychium spicatum* root and rhizome essential oil collected from the (CSIR-IHBT) farm.

Compounds	**[a]RI_{cal}**	**[b]RI_{lit}**	**% Oil content G8-Rhizome (Mean ± SE)**	**% Oil content G8-Root (Mean ± SE)**	**Mode of Identification**
α-thujene	930	931	0.1 ± 0.00	-	KI, MS
α-Pinene	939	939	1.4 ± 0.01	1.4 ± 0.02	KI, MS
α-Fenchene	956	951	0.1 ± 0.00	-	KI, MS
Camphene	958	953	-	1.0 ± 0.01	KI, MS
Sabinene	977	976	0.2 ± 0.01	0.4± 0.00	KI, MS
β-Pinene	983	980	2.8 ± 0.02	3.7 ± 0.04	KI, MS
β-myrcene	991	991	-	0.9 ± 0.02	KI, MS
Limonene	1035	1031	1.2± 0.01	1.4 ± 0.01	KI, MS
1,8-Cineole	1038	1033	73.3± 0.35	42.8 ± 0.41	KI, MS ^{13}C NMR
γ-Terpinene	1063	1062	0.1 ± 0.01	-	KI, MS
Linalool	1106	1098	1.3 ± 0.03	0.8 ± 0.00	KI, MS ^{13}C NMR
Camphor	1153	1143	-	0.3 ± 0.00	KI, MS
4-Terpineol	1187	1177	1.1 ± 0.01	1.8± 0.01	KI, MS ^{13}C NMR
α-Terpineol	1199	1189	1.6 ± 0.04	-	KI, MS
Bornyl acetate	1287	1285	0.1 ± 0.00	-	KI, MS
Copaene	1379	1376	0.1 ± 0.00	-	KI, MS
trans-Caryophyllene	1424	1418	0.1 ± 0.02	0.3 ± 0.00	KI, MS
Aromadendrene	1453	1439	-	0.4 ± 0.00	KI, MS
α-Humulene	1455	1454	0.2 ± 0.00	-	KI, MS
Farnesene <(E)-β->	1457	1458	-	14.8± 0.02	KI, MS ^{13}C NMR
Germacrene D	1485	1480	0.3 ± 0.00	-	KI, MS
α-Muurolene	1501	1499	0.8 ± 0.03	1.2 ± 0.01	KI, MS
γ-Cadinene	1517	1513	0.4 ± 0.01	-	KI, MS
Selinene (7-epi- α)	1521	1517	-	0.6 ± 0.00	KI, MS
δ-Cadinene	1522	1524	0.3 ± 0.11	-	KI, MS ^{13}C NMR
Elemol	1556	1549	1.0 ± 0.00	1.4 ± 0.01	KI, MS

Nerolidol E	1567	1564		0.3 ± 0.04	KI, MS
Carryophyllene oxide	1588	1581	1.6 ± 0.03	-	KI, MS
Humulene epoxide	1613	1606	0.2 ± 0.00	-	KI, MS
10-epi-γ-Eudesmol	1615	1619	-	0.6 ± 0.07	KI, MS
γ-Eudesmol	1639	1630	0.2 ± 0.05	4.3 ± 0.05	KI, MS
t-Muurolol	1653	1645	1.4 ± 0.02	-	KI, MS
Agarospirol	1655	1646	-	1.2 ± 0.01	KI, MS
α -Cadinol	1666	1653	0.6 ± 0.01	-	KI, MS
Eudesmol (7-epi-alpha)	1671	1662	5.4 ± 0.09	-	KI, MS ^{13}C NMR
Bulnesol	1673	1666	-	9.5 ± 0.11	
Total [%]			95.9	89.1	
Monoterpene hydrocarbons			7.1	8.8	
Oxygenated monoterpenes			76.2	45.7	
Sesquiterpene hydrocarbons			2.2	17.3	
Oxygenated sesquiterpenes			10.4	17.3	

[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).

SE: Standard error

[c]Compound confirmed using ^{13}C NMR (Ferreira et al., 1998; Kubeczka and Formacek, 2002).

In this study, roots and rhizomes were contained highest 1,8-cineole in the cultivated species of *H. spicatum.* Earlier reported studies have also detected 1,8-cineole as main oil component in *H. spicatum* (19.8-66.9%) (Koundal et al., 2015; Sabulal et al., 2007; Verma and Padalia, 2010). We had also observed 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.

Our study revealed that qualitative and quantitative chemical variations were found in root and rhizome part of cultivated species of *H. spicatum.* Rhizome is the good source of 1,8-cineole and 7-epi-α-eudesmol while root are the source for obtaining 1,8-cineole, (E)-β-farnesene and bulnesol in major concentration.

In earlier reported studies on analysis of EOs constituents of *H. spicatum* rhizomes and roots displayed the presence of components, 1,8-cineole, α-pinene, limonene, p-cymene, myrcene, β-pinene, τ-muurolol, α-eudesmol, linalool, 4-terpineol, 10-epi-γ-eudesmol and α-terpineol as major volatile constituents (Garg et al., 1977; Sabulal et al., 2007; Joshi et al., 2008; Verma and Padalia, 2010). 1,8-Cineol utilized as reduced severity of dyspnea and improvement of health status. Cineol was also used in treatment of COPD (chronic obstructive pulmonary disease) patients (Worth et al., 2009). Also 1,8-cineol widely used asantibacterial, repellent, toxicant, grain protectant, culinary purposes and in pharmaceutical industry, as a food additive (Obeng-Ofori et al., 1997; Sebei et al., 2015; Jaliazadeh-amin and Maham, 2015).

4 Discussion

After the perusal of parameters as shown in Table 1 to 3, it was revealed that the application of growth regulators in *H. spicatum* significantly affected germination percentage, plant height and root length. However, the germination percentage was not statistically significant. Similar non- significant effect of NAA and GA3 have been reported Bhattacharya et al. (1995) in *Pelargonium graveolans.* Since last 10 years, various R and D trials are going on different aspect on *Hedychium* crop in the institute as this crop is perennial and deciduous.

The crop of *Hedychium* can be raised both by seeds and rhizomes, but, crop raised by seed takes more time (4-5 years), for the formation of mature marketable rhizomes. Therefore, it is always better to raise the crop from rhizomes (Rana et al., 2004). Generally, this crop was propagated only by rhizomes for its immediate commercial value. Owing to this, the crop in its natural habitat has become endangered and may be extinct very soon. The annual crop cycle has been completed in 6 months only. The flower and seed germination were also recorded in every year. However, there is no seed germination or very few; hardly 2 to 3 % in natural climatic conditions. Having this experience, we conducted this experiment to study the seed performance in terms of germination. The highest germination was only 32.33 percent. The lower concentrations produced inhibitory effect on seed germination, while higher concentrations have produced stimulatory effect on seed germination. It also reveals that there is a trend in germination percentage, statistically significant shoot number and in root length from lower concentration to higher concentrations (Tables 1-3). It is clearly evident that the crop time is fixed, in it all physiological and metabolically processes. In 2004, the germination was only in July month and it has not extended beyond July. In the repeat trial in 2005, germination percentage showed an increasing trend from April to June, and thereafter, no further germination was recorded. It means the crop cycle is fix and saturated in on its all internal, physiological and metabolically processes. Similar pattern has been reported in case of *Aconitum balfourii* seed as the BAP and zeatin riboside did not enhance seed germination and has produced inhibitory effect on *Aconitum heterophyllum* due to lower concentration of BAP (Thapliyal and Thapliyal, 2005). It may be assumed that growth regulators treatments of seeds supplemented the endogenous level of hormones level in increased its availability in the surface area, which resulted and assist in seed germination. It also depends upon the endogenous hormones status or nutrients availability, enzymes and mobilization of foodsmaterial leading to cell division, cell elongation and successful seed germination. It has reported same type of results on seed germination Khan (1980). It is clear that higher concentration of IAA, IBA and NAA have produced promontory effect on seed germination of *H. spicatum.*

During both the years, growth hormones had effect on number of plantlets per plot (Table 2). Rhizomes treated with GA3 250 mg/l recorded lower number of plantlets and rhizomes treated with BAP 25 mg/l recorded higher number of plantlets in comparison to rhizomes treated with other concentrations of the same growth hormones. However, control rhizomes recorded slightly lesser number of plantlets in comparison to rhizomes treated with BAP 25 mg/l. In both the years, the growth hormones produce stimulatory effect on number of leaves per plants (Table 2). In the case of BAP 25 mg/l, maximum number of leaves was recorded per plant followed by control. While in GA3 250 mg/l minimum number of leave were obtained per plant.

The growth hormones treatments significantly influenced the yield of fresh rhizomes of *H. spicatum*. The highest yield of rhizome was recorded in BAP 25 mg/l which is almost double to that of control (Table 2), while the lowest yield was recorded in GA3 250 mg/l.

It is, thus, evident from above data that rhizomes treated with BAP 25 mg/l showed the best results whereas results shown by the rhizomes treated with GA3 250 mg/l is not upto the mark. The higher concentrations of GA3 produced inhibitory effect on overall parameters. It is proposed that treatment of rhizome with plant growth regulators supplemented the endogenous level of hormones and increased its availability in the surface area or cut ends of the rhizomes, which resulted and assist in rhizome sprouting. Although the promontory effect of hormones during root formation is well documented (Haissag, 1986), however, it is not known if only the BAP, GA3 and IAA participate in rhizome initiation or they are oxidized or conjugated with other endogenous compounds before their active participation in physiological action. However, it is confirmed from the results that BAP promote rhizome initiation, other growth parameters and increased the overall yield of rhizome after two years in comparison to other used hormones and control. It

may be assumed that if we use the lower concentration of BAP 25 mg/l for dipping treatment of *H. spicatum* rhizome then the overall yield will be doubled.

Some compounds are already stored in *H. spicatum* rhizomes. These compounds function via activation of enzymes, mobilization of food material leading to cell division, cell elongation and successful growth of rhizomes. It has reported such types of studies in some seed germination. Thus, these findings are agreed with our results Khan (1980). It has defined that IAA & Kinetin is enhancing leaf number and plant height in their ability to induce cell division, cell elongation and chlorophyll synthesis Mukaila (1996). Stimulation of plant height, no. of leaves and side plantlets, rhizome yield and overall growth by BAP in low concentration also agreed with above findings. The possible reason for this could be that the lower concentration of BAP induce cell division, cell elongation and hence the enhancement of growth and ultimately the yield of the rhizome. It has been concluded that the higher concentrations of GA_3, BAP and IAA produced inhibitory effect on all parameters of *H. spicatum*.

In conclusion, roots and rhizomes essential oil composition of cultivated species of *H. spicatum* displayed 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 food additive, drug formulation and treatment of COPD patients. 1,8-Cineol recommended, especially due to the lack of relevant side effects and relatively low cost because COPD is an extremely costly disease and a cause of major financial and social burden. Our finding have great evidence for cultivated *H. spicatum* (CSIR-IHBT Palampur) contain highest concentration of 1,8-cineol as compare 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. We had observed that in our previous report on *H. spicatum* rhizomes essential oil. The content of 1,8-cineol was lower from present study. It happened because we had taken rhizomes along with roots and the crop maturity was two years. Thomas Sinjumol and Man (2016) has reported the chemical composition of *Hedychium forrestii*, these studies fully agreed our studies. We had found in present study that roots have less 1,8-cineol content. However in present study we had taken three years old rhizome which suggest that 1,8-cineol content in *H. spicatum* increses with the maturity of crop. Solanki et al. (2016) has emphasized its conservation importance in the natural habitat in Western Himalaya (forest), where its natural environment. As per our surveys in Western Himalaya since last fifteen years, it was reported that most of the natural clusters of its plantation has been extinct by illegal way of harvesting and pressure of unfair means by pharmaceutical industries. So in natural habitat this crop should be re-established and grow very well by the support of our Institute and forest department.

Acknowledgments

The authors acknowledge the CSIR to provide grant under the project BSC-110. Authors are also grateful to Director, CSIR-IHBT Palampur to provide constantly help and other essential requirements. Authors thank Shri Amit Kumar to provide help in editing the manuscript.

References

Adams RP. 2007. Identification of essential oil Components by Gas Chromatography / Mass Spectroscopy. No. Ed.4 pp.viii 804, Allured Publishing Corporation, Carol Steam. IL, USA

Bhatt VP, Negi V, Purohit VK. 2009. *Hedychium spicatum* Buch.-Ham. a high valued skin glowing and curing medicinal herb needs future attention on its conservation. Nature and Science, 7(9): 75-77

Bhattacharya AK, Rajeshwar Rao BR, Kaul PN, Singh K, Singh CP. 1995. Response of rose- scented geranium (*Pelargonium spp*.) to plant growth regulator. Indian Perfumer, 39(2): 99-101

Bisht GS, Awasthi AK, Dhole TN. 2006. *Antimicrobial activity of* Hedychium spicatum. Fitoterapia, 77: 240-242

Chatterjee SK. 2002. Cultivation of medicinal and aromatic plants in India- A commercial approach. Acta Horticulturae, 576: 191-202

Collett H. 1921. Flora Simlensis. A Handbook of the Flowering Plants of Shimla and the Neighbourhood. Thacker, Spink & Co, Dehradun, India

Ferreira MJP, Emerenciano VP, Linia GAR, Romoff P, Macari PAT, Rodirigues GV. 1998. *C NMR spectroscopy of monoterpinoids.* Progress in Nuclear Magnetic Resonance Spectroscopy, 33: 152-206

Garg SN, Shawl AS, Gulati BC. 1977. Essential oil of *Hedychium spicatum var*. *acuminatum*. Indian Perfumer, 2: 79-82

Ghildiyal S, Gautam MK, Joshi VK, Goel RK. 2012. Pharmacognostical study of *Hedychium spicatum (Ham-Ex-Smith) Rhizome.* Asian and Pacific Journal of Tropical Disease, S148-S153

Giri D, Tamta S, Pandey A. 2010. A review account on medicinal value of *Hedychium spicatum Buch-Ham ex Sm:* Vulnerable medicinal plant. Journal of Medicinal Plants Research, 4(25): 2773-2777

Haissag BE. 1986. Influences of auxins and auxin synergists of adventitious primordia initiation and development. New Zealand Journal of Forestry Science, 4: 311- 327

Jadhav V, Kore A, Kadam VJ. 2007. In-vitro pediculicidal activity of *Hedychium spicatum* essential oil. Fitoterapia, 78: 470-473

Jalilzadeh-Amin G, Maham M. 2015. The application of 1,8-cineole, a terpenoid oxide present in medicinal plants, inhibits castor oil induced diarrhea in rats. Pharmaceutical Biology, 53 (4): 594-599

Joshi B, Tyagi V. 2011. Traditional knowledge and utilization of medicinal plants of Himalayan region. Nature and Science, 9: 5

Joshi S, Chanotiya CS, Agarwala G, Prakasha O, Panta AK, Mathela CS. 2008. Terpenoid compositions, and antioxidant and antimicrobial properties of the rhizome essential oils of different Hedychium species. Chemistry and Biodiversity, 5: 299-309

Kamboj VP. 2000. Herbal medicine. Current Science, 78: 35-39

Khan AA. 1980. The Physiology and Biochemistry of Seed Dormancy and Germination. North Holland Co, New York, USA

Koundal R, Rawat K, Agnihotri VK, Meena RL, Gopichand, Singh RD, Padwad YS. 2015. Temporal and spatial variation in quality of essential oil of *Hedychium spicatum* and evaluation of its antioxidant activity. Journal of Essential Oil Research, 27(3): 217-224

Kubeczka KH, Formacek V. 2002. Essential Oils Analysis By Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy. Wiley, New York, USA

Lu Y, Zhong CX, Wang L, Lu C, Li X.L, Wang PJ. 2009. Anti-inflammation activity and chemical composition of flower essential oil from *Hedychium coronarium*. African Journal of Biotechnology, 8(20): 5373-5377

Mishra T, Pal M, Meena S, Datta D, Dixit P, Kumar A, Meena B, Rana TS, Upreti DK. 2016. Composition and in vitro cytotoxic activities of essential oil of *Hedychium spicatum* from different geographical regions of western Himalaya by principal components analysis. Natural Product Research, 30: 1224-1227

Mukaila M, Muktar K, Agboola DA. 1996. Responses of some Nigerian vegetables to plant growth regulators treatments. Revista Biological Tropical, 44(3)/45(1): 23-28

Obeng-Ofori D, Reichmuth CH, Bekele J, Hassanali A. 1997. Biological activity of 1,8 cineole, a major component of essential oil of *Ucimum kenyense (Ayobangira)* against stored product beetles. Journal of Applied Entomology, 121: 237-243

Prakash A, Singh KK. 2001. Observation on some high valued ethno- medicinal plants among the tribals of Uttar Pradesh. Journal of Medicinal and Aromatic Plant Sciences, 22/4A and 23/1A: 519-552

Prakash B, Singh P, Kedia A, Dubey NK. 2012. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and in vivo efficacy in food system. Food Research International, 49: 201-208

Prakash O, Rajput M, Kumar M, Pant AK. 2010. Chemical composition and antibacterial activity of rhizome oils from *Hedychium coronarium Koenigand Hedychium spicatum Buch-Ham.* Journal of Essential Oil. Bearing Plants, 13(2): 250-259

Rajasekaran K, Sakhanokho HF, Tabanca N. 2012. Antifungal activities of Hedychium essential oils and plant extracts against mycotoxigenic fungi. Journal of Crop Improvement, 26: 389-396

Rana JC, Sharma BD, Jha BJ, Kumar M. 2004. Cultivation of *Hedychium spicatum* (Kapoor Kachari), *Valeriana wallichii* and *Roscoea purpurea* in hill regions of India. Indian Forester, 1008-1019

Rawat S, Bhatt ID, Rawal RS, Nandi SK. 2014. Effect of developmental stage on total phenolics composition and anti-oxidant activities in *Hedychium spicatum Buch.-Ham*. ex. D. Don. The Journal of Horticultural Science and Biotechnology, 89(5): 557-563

Sabulal B, George V, Dan M, Pradeep NS. 2007. Chemical composition and antimicrobial activities of the essential oils from the rhizomes of four *Hedychium* species from South India. Journal of Essential Oil Research, 19: 93-97

Sebei K, Sakouhi F, Herchi W, Khouja ML, Boukhchina S. 2015. Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biological Research, 48: 7

Solanki DJ, Chavda H, Makim R. 2016. A review on vulnerable plant Shati -*Hedychium spicatum*. International Journal of Ayurveda & Alternative Medicine, 4(6): 252-257

Stein SE. 1990. Mass Spectral Database and Software (Version 3.02). National Institute of Standards and Technology (NIST), Gaitherdurg, MD, USA

Stone R. 2002. Biological gets under the skin of plants and peers. Science, 296: 1597-1599

Thapliyal M, Thapliyal RC, 2005. Recent advances in research of seed technology of medicinal plants- Indian scenario. Journal of Applied Research on Medicinal and Aromatic Plants, 27: 320-327

Thomas Sinjumol and Man B. 2016. Chemical composition, antibacterial and antioxidant properties of essential oil from the rhizomes of *Hedychium forrestii* var. *palaniense* Sanoj and M. sabu. Indian Journal of Pharmaceutical Sciences, 78(4): 452-457

Verma RS, Padalia RC. 2010. Comparative essential oil composition of different vegetative parts of *Hedychium spicatum Smith.* from Uttarakhand, India. International Journal of Green Pharmacy, 292-295

Worth H, Schacher C, Dethlefsen U. 2009. Concomitant therapy with Cineole (Eucalyptole) reduces exacerbations in COPD: A placebo-controlled double-blind trial. Respiratory Research, 10: 69

Zhang WJ. 2017a. Network pharmacology of medicinal attributes and functions of Chinese herbal medicines: (I) Basic statistics of medicinal attributes and functions for more than 1100 Chinese herbal medicines. Network Pharmacology, 2(2): 17-37

Zhang WJ. 2017b. Network pharmacology of medicinal attributes and functions of Chinese herbal medicines: (II) Relational networks and pharmacological mechanisms of medicinal attributes and functions of Chinese herbal medicines. Network Pharmacology, 2(2): 38-66