

Research Article

Study of the Variability of the Chemical Profile, and Biological Activity Approaches of *Hedychium coronarium* J. Koenig Essential Oil from Different Habitats of Uttarakhand, India

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Received 5 January 2023; Revised 18 May 2023; Accepted 26 May 2023; Published 7 June 2023

Academic Editor: Said Gharby

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The objective of the current study was to evaluate the altitudinal variability in chemical compounds, nematocidal, insecticidal, herbicidal, and antifungal activity of the essential oils of *H. coronarium* collected from four different habitats of Tarai and Kumaun region of Uttarakhand. Essential oils were hydro-distilled from rhizomes of *H. coronarium*, and their yield varied from 0.05 to 0.25% (v/w). Based on GC-MS analysis, the major compounds observed were 1, 8-cineole (12.21–18.27%), coronarin E (10.20–14%), α -terpineol (5.32–10.13%), terpinen-4-ol (2.20–4.67%), α -pinene (4.90–8.77%), Z-caryophyllene (4.67–12.29%), and linalool (1.96–4.62%). To visually observe the variation in the oil composition profile of essential oils, heat map clustering, Venn diagram, and principal component analysis (PCA) were performed. To evaluate the pesticidal properties, the essential oils were tested under laboratory conditions. Additionally, significant nematocidal activity was demonstrated by essential oils against *Meloidogyne incognita*, good insecticidal activity against *Spodoptera litura*, and moderate herbicidal activity on *Raphanus raphanistrum* subsp. *sativus*, and effective antifungal activity against *Fusarium oxysporum* and *Curvularia lunata* was observed in the current investigation.

1. Introduction

Hedychium coronarium J. Koenig is a monocotyledon, perennial, aromatic herb grown throughout tropical and subtropical countries, i.e., India, Bangladesh, Brazil, China, Japan,

and South Asia [1]. In India, it is spread over Assam, Manipur, Peninsular, and Sikkim [2]. It is recognized as the “*gandasuli*” of India, a Sanskrit word that means “the princess or queen’s perfume.” It has fleshy, branching, and knotty rhizomes with attractive and fragrant flowers. *H. coronarium* is frequently

used in herbal folk and traditional medicine to treat asthma, arthritis, bronchitis, blood illnesses, eye diseases, gastric problems, and a variety of other diseases. It has long been used as a stimulant, febrifuge, and antirheumatic [3]. Almost all parts of this plant are utilized as medicinal as well as other daily uses, and its uses vary from region to region [4–6]. For instance, its leaves are useful for indigestion remedies, hypertension, and rigid and sore joints [5]. Similarly, rhizomes are extensively utilized as a food flavor and spice globally, especially in eastern parts of Asia. Additionally, piles bleeding, urinary tract stones, and irregular menstruation are also conditions that the rhizomes are used to cure [7]. In the Amarkantak district of Madhya Pradesh, India, its floral extract is used as an eye sedative and cures “*motiabind*” (cataract) [8]. The flowers and stems also stand for commercial importance as used in the manufacturing of body spray and paper and consumed as vegetables [9]. *Hedychium* essential oil (HOEO) has been found to possess number of biological activities such as antitumor, antiallergic, leishmanicidal, cytotoxic, antifungal, molluscicidal, antibacterial, analgesic, anti-inflammatory, and larvicidal actions [10–13]. Its rhizomes are also utilized as anticarcinogenic, antioxidant, antihypertensive, and antimalarial properties [14, 15].

HOEO contains a vast variety of bioactive compounds having an important role in pharmacology and perfumery. Compounds including linalool, α -pinene, β -pinene, β -caryophyllene, 1,8-cineole, and α -terpineol have been found to be the main constituents in HOEO [16, 17]. Chemo profile of *H. coronarium* essential oil attributable to variabilities in altitudinal and geographical locations such as varied composition was reported in its essential oils from Kumaun region, North India, Kerala region of South India, and from parts of Eastern India [11, 18, 19]. It has also been reported that *H. coronarium* and *H. coccineum* rhizome’s essential oil showed strong nematocidal properties against *Caenorhabditis elegans* and *Meloidogyne incognita* nematode [20, 21], remarkable antifungal activity against *Candida* and *Cryptococcus* [22], and significant herbicidal activity against lettuce seeds (*Lactuca sativa*). Similarly, the essential oil of *H. coronarium* was found to possess insecticidal activity against *Stephaniti spyrioides*, *Aedes aegypti*, and *Solenopsis invicta* [23].

To the best of our knowledge, no comparative study has been reported related to the chemical composition and pesticidal activities of *H. coronarium* essential oils from Kumaun Hills of India, reflecting the impact of geographic differences and ecological conditions. Therefore, this work aims to study the impact of different habitats and geographical variability on the phytochemical composition of HOEO from different localities in Tarai and Kumaun Hills (India) and to study the effect of these phytochemical variations on the nematocidal, insecticidal, herbicidal, and antifungal activities.

2. Materials and Methods

2.1. Plant Material. The plant material was collected from four different habitats of the Tarai and Kumaun region of Uttarakhand viz, Pantnagar, Bageshwar, Nainital, and Pithoragarh, in the month of October to November, 2020 (Table 1). The geographical localization of collection sites of

the plant material is represented in Figure 1. The plant material was identified by one of the authors (Dr. D.S. Rawat), plant taxonomist, Department of Biological Sciences, College of Basic Science and Humanities, Govind Ballabh Pant University of Agriculture and Technology (G.B.P.U. A. and T.), Pantnagar.

2.2. Essential Oil Isolation. Fresh rhizomes of the plant (about 1.5 kg) were subjected to a Clevenger-type apparatus for 3 hr to isolate the essential oils in about 1 L (2/3 of plant material) of water [24]. The obtained essential oils were dried over anhydrous sodium sulfate (Na_2SO_4) to remove water traces and then stored at a low temperature (4°C in refrigerator) for further analysis.

2.3. GC-MS Analysis. The chemical composition of essential oils was analyzed using gas chromatography mass spectroscopy (GC-MS) (PerkinElmer GCMS-SQ8 instrument) with PE5 (30.0 m \times 250 μm id, 0.25) column. 1 μL of essential oil was injected with the injector temperature set at 280°C . Helium was used as carrier gas and the flow rate was kept as 1 mL/min with a split ratio of 50.1. The initial temperature was set to 50°C for 3 min and increased up to 200°C with a ramp of $3^\circ\text{C}/\text{min}$ then $6^\circ\text{C}/\text{min}$ up to 250°C (isotherm for 2 min) and finally held for 11 min. Compounds of essential oils were identified by comparing their mass fragmentation pattern and relative retention index (RI) values with mass spectral library from the NIST (NIST version 2.1) and WILEY (7th edition) libraries and also by matching the fragmentation pattern of the mass spectral data with those described in the literature [25]. The experimental retention index was calculated by injecting a series of *n*-alkanes (C_7 – C_{20}). The compounds were quantified by measuring the peak area normalization and presented as the percentage of total peak areas. The percentage of chemical compound was calculated by the peak area normalization method.

2.4. Biological Activities

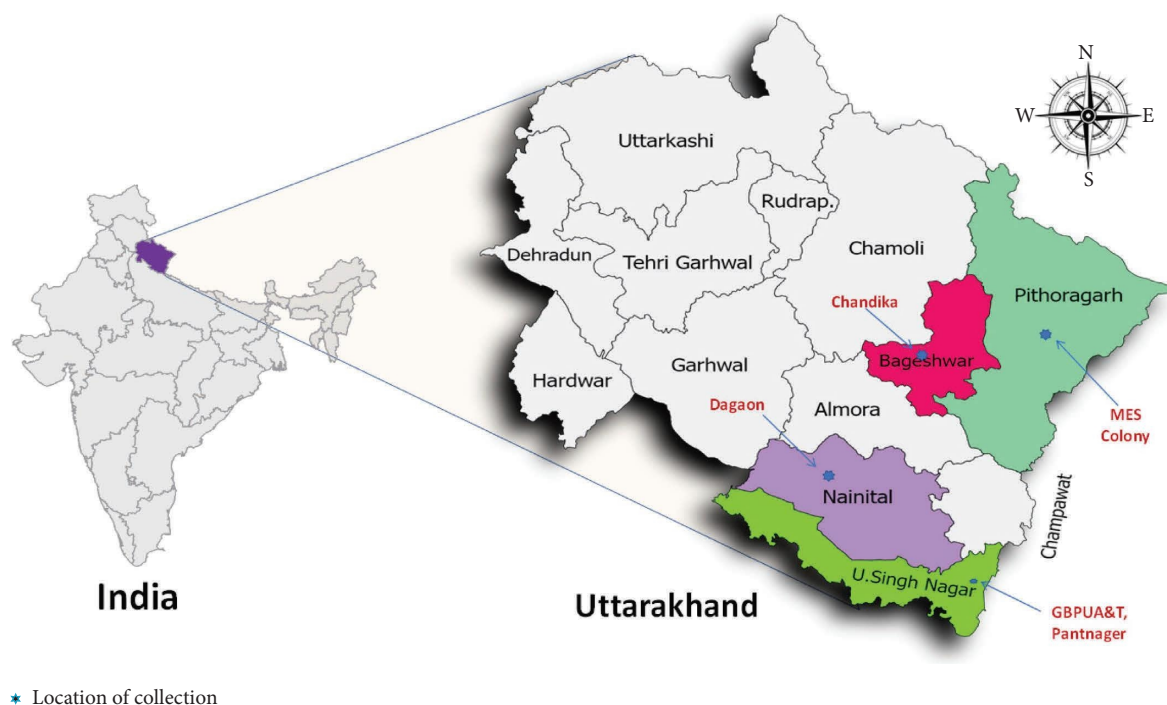
2.4.1. Nematocidal Activity

(1) *Nematode Population Collection.* *Meloidogyne incognita* eggs were collected from infected roots of tomato (*Solanum lycopersicum*). The infected roots were obtained from the Crop Research Center, G.B.P.U.A. and T., Pantnagar, and maintained in a glasshouse at a temperature of $25 \pm 2^\circ\text{C}$. Plants’ roots with galls were collected and washed with water to remove soil from diseased roots. Mature egg masses were hand-picked and cultured in distilled water at a temperature of 25°C . Freshly hatched second-stage larvae (J_2) were used for further trials.

(2) *In Vitro Nematode Mortality Bioassay.* Within 48 hr of hatching, about 100 second-stage juveniles per ml of water were taken into gridded petri dishes. Different essential oil concentrations (0.25, 0.5, and 1.0 $\mu\text{L}/\text{mL}$) in 1.0% Tween-20 water solution were used as treatments. The procedures were carried out three times, in random order. The juveniles in 1% Tween-20 water solution served as the control group. At 24,

TABLE 1: Details of plant materials collected from different geographical locations of Uttarakhand.

Essential oil samples	Site of collection	District, state	Latitude (N); longitude (E)	Elevation (m)	Time of collection	Voucher no.
HCPNO	G.B.P.U.A. & T., Pantnagar (cultivated)	Udham Singh Nagar, Uttarakhand	29°01'15.6" (N); 79°28'20.7" (E)	231	28 October 2020	GBPUH-1035
HCBO	Chandika (cultivated)	Bageshwar, Uttarakhand	29°51'01.7" (N); 79°46'29.4" (E)	998	17 November 2020	GBPUH-1037
HCNO	Dogaon (wild)	Nainital, Uttarakhand	29°19'19.9" (N); 79°30'17.3" (E)	1046	18 November 2020	GBPUH-1036
HCPO	MES colony (cultivated)	Pithoragarh, Uttarakhand	29°34'31.2" (N); 80°14'55.0" (E)	1505	23 October 2020	GBPUH-1034

FIGURE 1: Geographical localization of collection sites of *H. coronarium* from Kumaun and Tarai region of Uttarakhand.

48, 72, and 96 hr, nematodes were observed for mortality assay using a stereo binocular microscope at 4x magnification. Complete motionless nematodes were considered dead and taken out in distilled water for mortality confirmation. Abbott's formula, which is provided below, was used to measure percent mortality [26].

$$\text{Percent Mortality} = \frac{Mt - Mc}{100\% \text{ of initial population} - Mc} \times 100, \quad (1)$$

where Mt = mortality in treatment; Mc = mortality in control.

(3) *Egg Hatchability Bioassay*. Two egg masses of *M. incognita* were placed in gridded Petri plates containing essential oils samples at different concentrations (0.25, 0.5, and 1 $\mu\text{L}/\text{mL}$). The control group consisted of egg masses placed in a Tween-20 (1.0%) water solution. At a constant temperature ($27 \pm 1^\circ\text{C}$), each treatment was set up in the

BOD incubator in triplicates in an arbitrary order. For the durations of 24, 48, 72, and 96 hr, the hatching percentage of eggs was observed using a microscope at 4x magnification. Furthermore, the percent egg hatchability was calculated using the Abbott's formula as shown as follows:

$$\text{Percent egg hatching inhibition} = \frac{Mt - Mc}{100\% \text{ of initial population} - Mc} \times 100, \quad (2)$$

where Mt = egg hatching in treatment; Mc = egg hatching in control.

2.4.2. Insecticidal Activity

(1) *Test Insect*. Insecticidal activity was performed against the test insect, tobacco cutworm, *Spodoptera litura*. In addition to damaging tobacco crops, it also damages other crops such as castor, cotton, chilly, and tomato.

(2) *Collection of Larvae and Maintenance.* The egg mass of *S. litura* was collected from wild castor (*Ricinus communis*) plant, at the CRC (Crop Research Center), G.B.P.U.A. and T., Pantnagar, Uttarakhand, India. Insect rearing was done in a clean, muslin-covered plastic jar, and the optimal laboratory conditions were maintained for the test insect rearing at 27°C temperature and 75–80% humidity. Up until the fourth instar of the larval stage, test insects were fed with fresh castor leaves every day, and the fourth instar larvae were used in insecticidal activities.

(3) *Bioassay of Insecticidal Activity.* The insecticidal efficacy of *H. coronarium* rhizome essential oils was tested using the leaf dip method [27]. Briefly, cleaned castor leaves were cut into a 25 cm² section and dipped into varied concentrations of essential oil prepared in 1% Tween-20 aqueous solution. The leaf discs were slanted on blotting paper for two to three minutes before being placed in the Petri dish to drain the excess solution. After being starved for 12–24 hr, adult larvae of the fourth instar (five in total) were released in separate Petri dishes. An aqueous solution of Tween-20 (1%) was taken as the control. Using Abbott's formula, the mortality (%) was determined at 24, 48, and 72 hr after the treatment. Probit analysis was used to calculate the LC₅₀ value.

$$\text{Percent Mortality} = \frac{\text{Mt} - \text{Mc}}{100\% \text{ of initial population} - \text{Mc}} \times 100, \quad (3)$$

where Mt = mortality in treatment; Mc = mortality in control.

2.4.3. *Herbicidal Activity.* Various measures, including seed germination inhibition, shoot length, and root length inhibition, were used to evaluate the herbicidal action of essential oils on the receptor plant, radish, *Raphanus raphanistrum* subsp. *Sativus* seeds.

(1) *Herbicidal Bioassay.* The herbicidal potential of essential oils was assessed using the prescribed protocol reported earlier [28]. In brief, radish seeds were surface sterilized for 15 minutes in a 5% sodium hypochlorite solution. Ten sterilized seeds were placed on Petri plates and covered by bilayered filter papers. The plates were then filled with 2 mL of the tested sample at various concentrations and then incubated at 25°C with a dark/light ratio of 12/12 hr. Standard herbicide, Pendimethalin served as positive control whereas, Tween-20 (1.0%) water solution was used as a negative control. After 5 days of incubation, root and shoot length, as well as the percentage of seed germination,

were measured. The formulas used to calculate the root and shoots length and seed germination inhibition were as follows:

(a) Seed germination inhibition

$$\% \text{Inhibition of seed germination} = 100 \times \left(1 - \frac{\text{Gt}}{\text{Gc}}\right). \quad (4)$$

Here Gt-no. of seeds germination in treatment
Gc-no. of seeds germination in control.

(b) Shoot length inhibition

$$\% \text{Inhibition of shoot length} = 100 \times \left(1 - \frac{\text{St}}{\text{Sc}}\right). \quad (5)$$

Here St-shoot length in treatment
Sc-shoot length in control.

(c) Root length inhibition

$$\% \text{Inhibition of root length} = 100 \times \left(1 - \frac{\text{Rt}}{\text{Rc}}\right), \quad (6)$$

where Rt-root length in treatment
Rc-root length in control.

2.4.4. *Antifungal Activity.* Culture of two phytopathogenic fungi, *Fusarium oxysporum* and *Curvularia lunata*, was obtained from Department of Plant Pathology, College of Agriculture, G.B.P.U.A. and T., Pantnagar, India. Poisoned food protocol developed by Grover and Moore [29] was followed to test the essential oils against selected fungi. The phytopathogenic fungi were revived and cultured by placing the fungal colonies aseptically on petri dishes containing the Potato Dextrose Agar (PDA) media. The Petri plates were incubated at 26 ± 2°C for a week. Seven-day-old culture of test fungi was used to create the assay discs, which were then aseptically injected with various concentrations of essential oils (50, 100, 250, and 500 µL/mL) on prepared plates. Under the same circumstances, a control dish without essential oils was equipped. The growth on the control plate was measured up to the edge of the plate. Each fungus strain's percentage of radial growth inhibition was calculated concerning the control. Around the petri dish, there were millimeter-sized clear zones of mycelia growth inhibition, which were used to identify antifungal activity. The standard fungicide used was carbendazim, and the percentage of inhibition was determined by using McKinney's methodology [30].

$$\text{Percent inhibition} = 100 \times \left(X - \frac{X}{Y}\right), \quad (7)$$

where X = growth in control, Y = growth in treatment.

2.5. In-Silico Pass Prediction Study. For the prediction of activity spectra for substances (PASS), the major compounds from essential oils were selected based on their higher percentage content. The SMILES format of the selected compounds was used to predict and simulated to PASS online web app, which expects the probable activity (Pa) and probable inactivity (Pi) of any compound, and it is applied to “drug-like” substances [31]. Compounds with Pa > Pi are the only constituents measured for possessing a particular biological activity [32].

2.6. In-Silico ADMET Study. For analyzing the pharmacokinetics properties (absorption, distribution, metabolism, and excretion (ADME)) and studies, the structures of the selected major compounds were converted into their SMILES format and then estimated using the ADME tool using the Swiss ADME online server (<https://www.swissadme.ch/>), as described earlier [33]. Toxicity profile in terms of different levels of toxicity such as organ toxicity (hepatotoxicity), oral toxicity, and toxicological endpoints (cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity) was studied by using ProTox-II webserver (https://tox.charite.de/protox_II).

2.7. Statistical Analysis. All the trials were conducted in triplicates, and the results were presented as mean \pm standard deviation (SD). Two-way or three-way analysis of variance (ANOVA) followed by a Tukey’s multiple comparison test was performed to test mean differences of treatments using RStudio 2021.09.2. The p value <0.05 was considered to show a significant difference. OriginPro 2021 version 9.8.0.200 was used to perform principal component analysis (PCA) on chemical analysis of the essential oils under investigation to find the most significant feature in the dataset. Heat map clustering was performed by using an online freely available webserver and heat mapper (<https://heatmapper.ca/expression/>). Pearson’s correlation test was determined by using the Corplot function in RStudio2021.09.2.

3. Result and Discussion

An altitudinal change from mean sea level is a significant aspect influencing terrestrial as well as hilly areas’ ecosystems. Therefore, considerable alteration in altitude level and habitat conditions can bring about corresponding changes in relative humidity, temperature, available water, wind speed, sunlight duration, etc. As a result, changes in environmental conditions (i.e., abiotic factors) will change many eco-physiological reactions in plant bodies. Hence, it is predicted that shifting ecological niches will affect the constituents and content of volatiles [34]. In the present study, three cultivated and one wild population of *H. coronarium* were collected in October and November, 2020 from different habitats and from different altitudes ranging from 231 m to 1505 m. The hydro-distilled essential oils’ average yield was $0.15 \pm 0.01\%$, $0.15 \pm 0.01\%$, $0.05 \pm 0.01\%$, and $0.25 \pm 0.01\%$ for the plant material collected from Pantnagar,

Pithoragarh, Bageshwar, and Nainital, respectively. The chemical composition of essential oils was analyzed using the GC-MS technique. The results showed the presence of a total of 16, 17, 18, and 14 compounds which accounted for about 92.23%, 90.48%, 92.98%, and 97.83% of the total essential oil composition for HCPNO, HCBO, HCNO, and HCPO, respectively, as shown in Table 2.

Results depicted that all the essential oils were dominated by oxygenated monoterpenes. Among the chemical constituents, 1, 8-cineole was the main compound of all the tested essential oils in variable amounts (Table 2). Other identified main constituents of HCPNO were coronarin *E* (14.00%), α -terpineol (10.13%), α -terpinyl acetate (9.15%), α -pinene (6.88%), (*Z*)-caryophyllene (5.23%), terpinen-4-ol (4.36%), myrtenal (4.29%), and longiborneol (4.17%). In HCBO, coronarin *E* (11.01%), isopulegol (8.21%), α -terpineol (7.51%), (*Z*)-caryophyllene (7.17%), *cis*-dihydromayurone (7.07%), caryophyllene oxide (6.21%), α -pinene (5.66%), terpinen-4-ol (4.11%), α -terpinyl acetate (4.08%), and 3-octen-5-yne (3.18%) were the other identified major constituents. In HCNO, the main constituents identified beside 1, 8-cineole were rosfoliol (11.50%), coronarin *E* (11.1%), α -terpineol (8.61%), α -patchoulene (8.54%), *cis*-limonene oxide (5.70%), linalool (5.69%), and α -pinene (4.90%). Whereas, (*Z*)-caryophyllene (12.29%), coronarin *E* (10.20%), α -terpinyl acetate (8.85%), isopulegol (7.20%), α -pinene (5.66%), α -terpineol (5.32%), terpinen-4-ol (4.67%), linalool (4.62%), and *endo*-fenchol (3.48%) were the other identified dominant compounds of HCPO.

To compare the chemical constituents of all the essential oils, a Venn diagram was generated (Figure 2). It represents that there is total of 7, 7, 6, and 2 compounds which were found unique in HCPNO, HCBO, HCNO, and HCPO, respectively. A total of 7 compounds (α -pinene, 1, 8-cineole, linalool, terpinen-4-ol, α -terpineol (*Z*)-caryophyllene, and coronarin *E*) were found common in all the investigated essential oils. It was observed that only β -atlantol was found common among all the tested essential oils except HCPNO. Whereas, only δ -2 carene was found common in HCPNO, and HCNO. α -Terpinyl acetate was present in both HCPNO and HCBO whereas, isopulegol was common in HCBO and HCPO. α -Patchoulene, α -muurolene, and rosfoliol were common in HCNO and HCPO. Based on the chemical profiling of essential oils, it was found that there is a significant variation in chemical composition in the essential oils. It is inferred that the variation may be due to altitude gradient, geographical dissimilarity, environmental, and climatic conditions, etc.

In addition, to visually observe the variation in the composition of different essential oils, heat map clustering was performed (Figure 3(a)). In the heat map clustering diagram, the distribution of traits was identified by colors, where red color showed the maximum value of the trait, while green color represents the minimum value of the traits. It can be seen that all the samples were divided into two main clusters based on their chemical composition. In the first cluster (A), two species viz., HCPNO and HCBO are there. Whereas, HCNO and HCPO are in the second cluster (B).

TABLE 2: Comparative chemical composition (in percent values) of *H. coronarium* essential oils from different locations.

S. no.	Compound	RI ^{lit.} value	RI ^{cal.} value	HCPNO (231 m)	HCBO (998 m)	HCCO (1046 m)	HPCO (1505 m)
1	2-Methyl-1,3-cyclohexadiene (H)	770	—	—	—	0.69 ± 0.05	—
2	2,3-Dimethyl-pyrazine	920	927	—	—	1.20 ± 0.01	—
3	α-Pinene (MH)	939	939	6.88 ± 0.14	—	4.90 ± 0.01	8.77 ± 0.05
4	α-Fenchene (MH)	952	952	2.20 ± 0.01	5.66 ± 0.04	—	—
5	Camphene (MH)	954	952	—	2.22 ± 0.01	—	—
6	Thuja-2,4(10)-diene (MH)	960	957	2.10 ± 0.01	—	—	—
7	3-Octen-5-yne (MH)	965	971	—	3.18 ± 0.04	—	—
8	δ-2 Carene (MH)	1013	1011	1.75 ± 0.01	—	1.04 ± 0.01	—
9	p-Cymene (MH)	1023	1023	—	—	1.54 ± 0.01	—
10	1,8-Cineole(OM)	1031	1032	18.27 ± 0.01	12.21 ± 0.14	16.20 ± 0.03	18.24 ± 0.03
11	Linalool(OM)	1096	1098	2.38 ± 0.01	1.96 ± 0.01	5.69 ± 0.01	4.62 ± 0.05
12	endo-fenchol (OM)	1116	1113	—	—	—	3.48 ± 0.01
13	trans-mentha-2,8-dien-1-ol (OM)	1137	1137	—	—	1.96 ± 0.02	—
14	cis-limonene oxide (OM)	1136	1138	—	—	5.70 ± 0.03	—
15	3-Methyl-2-isobutyl-pyrazine	1137	1134	—	—	—	—
16	Isopulegol (OM)	1149	1146	—	8.21 ± 0.02	—	1.98 ± 0.02
17	Isoborneol (OM)	1160	1156	2.03 ± 0.01	—	—	7.20 ± 0.05
18	2,6-Dimethyl aniline	1167	1167	—	—	1.88 ± 0.01	—
19	Terpinen-4-ol (OM)	1177	1177	4.36 ± 0.01	4.11 ± 0.01	2.20 ± 0.02	4.67 ± 0.14
20	α-Terpineol (OM)	1188	1189	10.13 ± 0.03	7.51 ± 0.01	8.61 ± 0.01	5.32 ± 0.05
21	Myrtenal (OM)	1195	1193	4.29 ± 0.02	—	—	—
22	Isobornyl acetate (OM)	1285	1290	—	2.73 ± 0.02	—	—
23	Bornyl acetate (OM)	1286	1285	—	0.71 ± 0.01	—	—
24	3,4,5,5-Tetramethyl-1,3 cyclopentadiene carboxylic acid (OM)	1288	1293	2.61 ± 0.05	—	—	—
25	α-Terpinyl acetate (OM)	1349	1349	9.15 ± 0.02	4.08 ± 0.01	—	—
26	(Z)-Caryophyllene (SH)	1418	1418	5.23 ± 0.01	7.17 ± 0.02	4.67 ± 0.01	4.67 ± 0.03
27	α-Patchoulene (SH)	1456	1450	—	—	8.54 ± 0.01	8.54 ± 0.02
28	α-Muurolene (SH)	1500	1499	—	—	2.04	2.04 ± 0.04
29	Caryophyllene oxide (OS)	1583	1581	—	6.21 ± 0.03	—	—
30	cis-dihydromayurone (OM)	1595	1597	—	7.07 ± 0.03	—	—
31	Longiborneol (OS)	1599	—	4.17 ± 0.05	—	—	—
32	Rosifolol (OS)	1600	1599	—	—	11.50 ± 0.04	11.50 ± 0.03
33	β-Atlantol (OS)	1608	1608	—	1.27 ± 0.04	1.42 ± 0.01	1.42 ± 0.02
34	5-Ethyl-2-methyl-pyridin-4-amine	1608	—	2.68 ± 0.03	—	—	—
35	Caryophylla-4(12),8(13)-dien-5à-ol (OS)	1640	1639	—	5.17 ± 0.01	—	—
36	Coronarlin E (OD)	2166	2166	14.00 ± 0.04	11.01 ± 0.05	13.20 ± 0.04	13.20 ± 0.05
<i>Class composition</i>							
Hydrocarbons (H)							
Monoterpene hydrocarbons (MH)							
Oxygenated monoterpene (OM)							
Sesquiterpene hydrocarbons (SH)							
Oxygenated sesquiterpene (OS)							
Oxygenated diterpene (OD)							
Others							
Total (%)							
			92.23	90.48	92.98	97.83	

RI^{lit.} = Literature retention indices value in reference (Adams) [25], RI^{cal.} = retention index on the PE-5MS column, calculated using homologous series of C₇-C₂₀ alkanes. HCPNO = *H. coronarium* essential oil from Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCCO = *H. coronarium* essential oil from Dogaon, Nainital, HPCO = *H. coronarium* essential oil from MES colony, Pithoragarh.

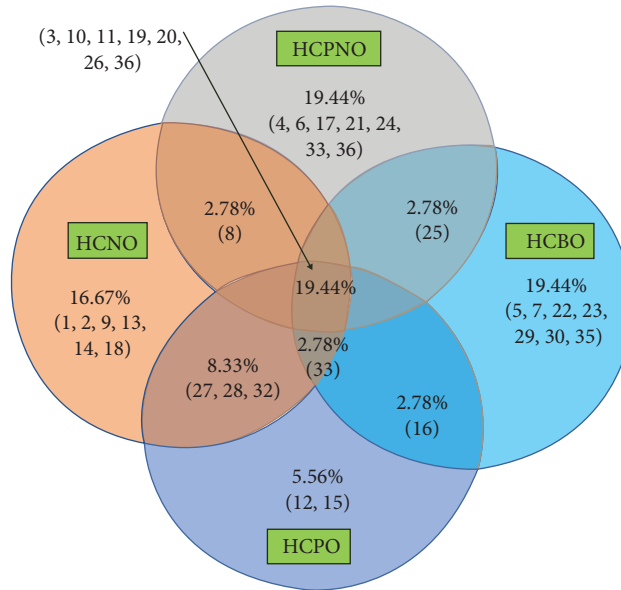


FIGURE 2: Venn diagram of the chemical composition of *H. coronarium* essential oils from different locations. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

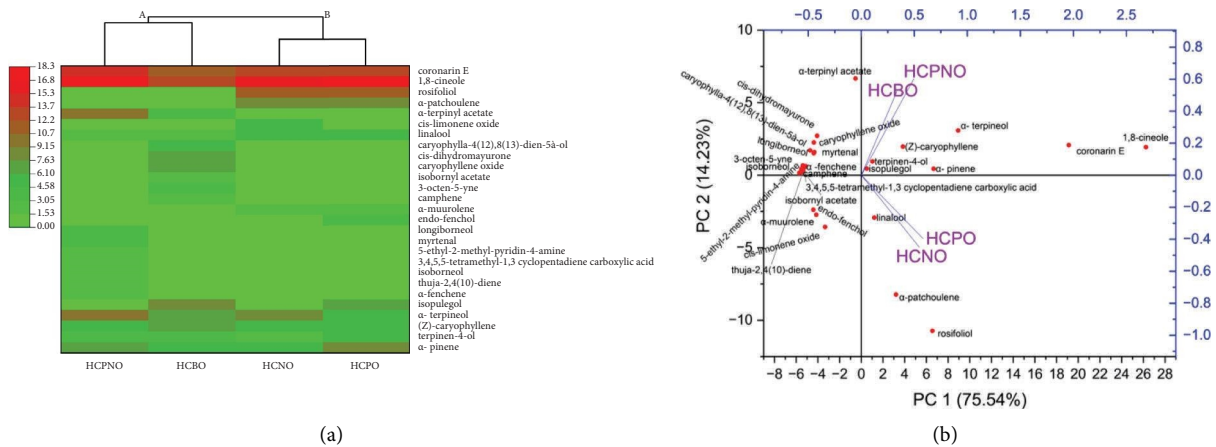


FIGURE 3: (a) Heat map clustering of *H. coronarium* essential oils, based on their essential oil compounds. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh). (b) PCA of *H. coronarium* essential oils, based on their essential oil compounds. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

Furthermore, to find the most significant features in the data, principal component analysis (PCA) is a well-known multivariate statistical technique for identifying the dataset's most key aspects (34). A PCA pattern was utilized to assess the phytochemical variability in the composition (>2.0%) of the essential oils from different habitats. Chemical compositional differences have a collective contribution rate of variance of only the first two principal components (PC1 and PC2) produced from the PCA method of 89.7%, which may explain much of the variance information (Figure 3(b)). The total compositional heterogeneity in the essential oils was thus characterized by

these two PCs. PC1 had an overall variance contribution of 75.5% and a positive correlation with linalool, α -patchoulene, and rosfoliol. Whereas, the influence of PC2 on the variance is 14.2% which was positively associated with 1,8-cineole, α -terpineol, terpinen-4-ol, coronarin E, (Z)-caryophyllene, α -pinene, and isopulegol.

Based on chemical profiling (a Venn diagram, heat map clustering, and PCA) of essential oils, it was found that there is a significant variation in chemical composition in the essential oils. It is inferred that the variation may be due to altitude gradient, geographical dissimilarity, environmental, and climatic conditions, etc.

The chemical composition of essential oils in present investigation was significantly different from recently reported essential oil of *H. coronarium* rhizome from Bhadrak district in Odisha [35]. However, the results were similar in comparison to some other studies. 1, 8-Cineole, an oxygenated monoterpene, found to dominate in presently investigated oils was also reported in a previous study on *H. coronarium* essential oils. For instance, in rhizome oils of *H. coronarium*, 1, 8-cineole was reported as the major constituent [10, 22]. The other compounds such as α -pinene, β -pinene, linalool, γ -terpinene, coronarin E, and 10-*epi*- γ -eudesmol were also observed as the main constituents in the rhizome essential oil of *H. coronarium* collected from 10 different locations of Eastern India [19]. Similarly, other studies also revealed the dominance of compounds such as 1, 8-cineole, β -pinene, and linalool in the rhizome part essential oils of other *Hedychium* species collected from the southern part of India [11, 36]. Whereas, β -pinene was found to be the most abundant compound in *H. coronarium* rhizome collected from Bhimtal, Uttarakhand [19]. Moreover, Joshi et al. [37] reported the presence of limonene, *p*-menth-1-en-8-ol, γ -terpinene, *trans-meta*-mentha-2, 8-diene, camphene, α -pinene, linalool, 10-*epi*- γ -eudesmol, and γ -curcumene as major compounds present in rhizome essential oil of the *H. coronarium* from three different collection sites viz., Jageshwar, Shimla, and Bhowali. Compounds such as 1, 8-cineole, linalool, α -pinene, α -terpineol, caryophyllene, and *p*-cymene have been also found in members of other plant families including lamiaceae and asteraceae family [38, 39], which shows the chemotaxonomic significance of the genus *Hedychium*. Comparing the results of the present study with previous reports from Uttarakhand, India, and other geographical regions worldwide, we found that there were qualitative and quantitative differences in phytochemical composition.

3.1. Biological Activities

3.1.1. Nematicidal Activity

(1) *Nematode Mortality Bioassay*. At various concentrations (0.25, 0.5, and 1.0 $\mu\text{L/mL}$), the nematicidal activity of essential oils was evaluated against second-stage juveniles of *M. incognita* for the duration of 24, 48, 72, and 96 hr. Findings revealed that nematode mortality was dose as well as time-dependent. At 1.0 $\mu\text{L/mL}$ dose level after 96 hr, HCBO was found to be most active, inhibiting larval mobility by 59.33%. The order of nematicidal activity of essential oils at the highest dose level (1.0 $\mu\text{L/mL}$) was as HCBO (59.33%) > HCPO (47.66%) > HCNO (47.33%) > HCPNO (42.33%). The percent mortality for tested essential oils has been depicted in (Table 3). The LC₅₀ values of the essential oils at different times of exposure are shown in (Table 4).

(2) *Nematode Egg Hatchability Bioassay*. Egg hatching was found to be strongly inhibited in a concentration as well as time-dependent manner. The rate of egg hatching was inversely correlated with essential oil concentration and directly correlated with exposure time. After 96 hr, the maximum rate of

egg hatching was recorded for HCNO (82.00%) at a minimum dose level (0.25 $\mu\text{L/mL}$) whereas the minimum rate of egg hatching was found in HCPO (15.66%) at the highest dose level (1.0 $\mu\text{L/mL}$) (Table 5). The IC₅₀ values were found to increase with the increase in exposure time (Figure 4).

Previous studies have shown the nematicidal activity of essential oil from the rhizomes of *H. coronarium* against *M. incognita* and *Caenorhabditis elegans* [20, 40]. According to Oka et al. [41], essential oils are a combination of various substances that in the situation of a nervous system disturbance, it may impair the nematode metabolism during the embryonic period as well as the mechanisms of movements. Andres et al. [42] claim that the compounds in essential oils interact with the cytoplasmic membrane and may change or harm the structure of polysaccharides, lipids, and phospholipids. Additionally, it is thought that the essential oils' nematicidal effects must be attributable to the phenols, aldehydes, and alcohols that oxidize these membranes. Based on the results observed in the present study, we inferred that the reduction of the egg-hatching process and high mortality of *J*₂ of *M. incognita* might be associated with the major and/or minor chemical constituents of the essential oils.

3.1.2. *Insecticidal Activity*. The insecticidal activity of different essential oils was tested against *Spodoptera litura* insect employing the leaf dip method. Results revealed the percentage of insect mortality was increased with exposure time (24, 48, and 72 hr) and concentration (10–100 $\mu\text{L/mL}$). At 72 hr, it was found that HCBO was most effective at a dose level of 100 $\mu\text{L/mL}$, with 59.33% larval mortality. The maximum percent mortality was observed in the order of HCBO (59.33%) > HCPO (47.66%) > HCNO (47.33%) > HCPNO (42.33%) at 100 $\mu\text{L/mL}$ concentration (Table 6). The LC₅₀ values of essential oils are presented in Table 7. Bruni et al. [43] investigated the insecticidal activity of *H. spicatum* essential oil against the larvae of the diamondback moth, *Plutella xylostella* (L.). Similarly, Sakhanokho et al. [23] and Koundal et al. [44] evaluated the insecticidal activity of *Hedychium* species rhizomes essential oils against insects such as *Stephanitis pyrioides*, *Aedes aegypti*, and *Solenopsis invicta*. They concluded that the toxic effects of essential oils on the test insect may be caused by a variety of bioactive compounds present in the oils, such as 1, 8-cineole, linalool, α -pinene, β -pinene, and (*E*)-nerolidol, or it may be the result of the synergistic effects of multiple compounds. These compounds might also be responsible for the insecticidal activity of essential oils under investigation; as such compounds are also present in variable amounts in the tested essential oils.

3.1.3. Herbicidal Activity

(1) *Inhibition of Seed Germination*. Table 8 shows the mean % seed germination inhibition by *H. coronarium* rhizome's essential oils at various doses (50–200 $\mu\text{L/mL}$). Results revealed that the essential oils revealed good to moderate herbicidal activity in a dose-dependent manner. The percent seed germination inhibition by tested essential oils was observed in the following order: HCPO (96%) > HCPNO

TABLE 3: Effect of essential oils on mortality percentage of second stage juveniles (J_2 s) of *M. incognita*.

Sample	Concentration ($\mu\text{L}/\text{mL}$)	Percent mortality (mean \pm SD)			
		24 hr	48 hr	72 hr	96 hr
HCPNO	0.25	20.33 \pm 0.57 ^t	21.66 \pm 0.57 ^{nopq}	26.66 \pm 1.52 ^{pqr}	31.33 \pm 0.57 ^{klmnop}
	0.5	25.33 \pm 0.57 ^{qrs}	28.00 \pm 1.00 ^{klmno}	29.33 \pm 1.15 ^{mnoqp}	34.33 \pm 2.08 ^{ijkl}
	1	30.66 \pm 1.15 ^{lmnop}	35.66 \pm 0.57 ^{hijk}	38.00 \pm 1.00 ^{ghi}	42.33 \pm 0.57 ^{efg}
HCBO	0.25	21.33 \pm 0.57 st	28.33 \pm 0.57 ^{mnoqp}	46.00 \pm 1.73 ^{cde}	48.66 \pm 1.15 ^{bc}
	0.5	27.00 \pm 1.73 ^{opq}	33.00 \pm 1.73 ^{jklm}	48.66 \pm 1.15 ^{bc}	52.00 \pm 2.00 ^b
	1	29.00 \pm 1.15 ^{mnoqp}	38.66 \pm 2.08 ^{fghi}	51.66 \pm 1.52 ^b	59.33 \pm 1.15 ^a
HCNO	0.25	19.33 \pm 1.15 ^t	28.66 \pm 1.00 ^{nopq}	31.66 \pm 0.57 ^{klmno}	35.66 \pm 2.08 ^{hijk}
	0.5	22.00 \pm 2.64 ^{rst}	31.66 \pm 0.57 ^{lmnop}	38.66 \pm 0.57 ^{fghi}	42.33 \pm 2.51 ^{efg}
	1	28.33 \pm 2.88 ^{mnoqp}	35.66 \pm 0.57 ^{klmn}	40.00 \pm 0.57 ^{fg}	47.33 \pm 2.08 ^{bcd}
HCPO	0.25	18.33 \pm 0.57 ^t	29.66 \pm 0.57 ^{lmnopq}	37.66 \pm 0.57 ^{ghij}	39.66 \pm 0.57 ^{gh}
	0.5	28.00 \pm 1.00 ^{nopq}	32.33 \pm 2.51 ^{klmn}	40.33 \pm 2.08 ^{fg}	40.66 \pm 1.15 ^{fg}
	1	35.66 \pm 0.57 ^{hijk}	39.00 \pm 1.00 ^{fghi}	43.33 \pm 1.52 ^{def}	47.66 \pm 0.57 ^{bcd}
Control	Water	0.00 \pm 0.00	0.66 \pm 0.57	1.66 \pm 2.08	3.33 \pm 1.52

SD = standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh.

TABLE 4: LC_{50} values of essential oils for nematode mortality against J_2 s of *M. incognita*.

Sample	Hr	* LC_{50} (%)
HCPNO	24	3.63 \pm 0.07 ^{bc}
	48	2.55 \pm 0.02 ^c
	72	3.04 \pm 0.05 ^{bc}
	96	2.52 \pm 0.03 ^c
HCBO	24	2.26 \pm 0.24 ^c
	48	3.29 \pm 0.07 ^{bc}
	72	4.37 \pm 0.33 ^{bc}
	96	6.08 \pm 0.08 ^{ab}
HCNO	24	3.16 \pm 0.03 ^{bc}
	48	3.51 \pm 0.02 ^{bc}
	72	3.23 \pm 0.04 ^{bc}
	96	2.57 \pm 0.01 ^c
HCPO	24	8.40 \pm 0.02 ^a
	48	8.58 \pm 0.02 ^a
	72	4.41 \pm 0.08 ^{bc}
	96	1.79 \pm 0.01 ^c

* LC_{50} = Lethal concentration; HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh.

(92.33%) > HCBO (90.37%) > HCNO (90.00%). The IC_{50} values were calculated to compare the relative herbicidal activities of all the tested essential oils as shown in Figure 5.

(2) *Inhibition of Root Length*. Based on percent root length inhibition (Table 9), IC_{50} values were also calculated at the time when 100% growth was attained in the control. The order of IC_{50} values was HCPO (194.58 \pm 2.57 $\mu\text{L}/\text{mL}$) > HCPNO (102.71 \pm 5.65 $\mu\text{L}/\text{mL}$) > HCNO (90.46 \pm 6.39 $\mu\text{L}/\text{mL}$) > HCBO (88.22 \pm 2.84 $\mu\text{L}/\text{mL}$) (Figure 5).

(3) *Inhibition of Shoot Length*. The shoot length inhibition at the highest concentration of essential oils (200 $\mu\text{L}/\text{mL}$) was observed in the following order: HCPO (100.00%) > HCBO (97.33%) > HCPNO (94.00%) > HCNO (93.66%) (Table 10). The lowest IC_{50} value was obtained for HCPNO

(94.00 \pm 1.00 $\mu\text{L}/\text{mL}$), whereas the highest IC_{50} value was observed for HCBO (97.90 \pm 3.41 $\mu\text{L}/\text{mL}$) (Figure 5).

The high amount of 1, 8-cineole might be responsible for the herbicidal activity of the tested samples as it is reported to have high toxicity against weed seeds in the previous study [45]. Several researchers suggested that essential oils and their pure compounds induced loss of membrane integrity by the generation of reactive oxygen species, affect mitochondrial respiration, effect on proline accumulation, inhibition on photosynthesis process, inhibition of DNA synthesis, and mitosis in weeds [46–48]. Rawat et al. [49] investigated the herbicidal activity of *H. spicatum* essential oil against *Raphanus raphanistrum*. They suggested that bioactive compounds (1, 8-cineole, curdione, isoborneol, (+)-linalool, germacrene D, (–)-borneol, camphene, (*E,E*)-germacrone, (+)- α -terpineol, camphene hydrate, α -pinene,

TABLE 5: Effect of essential oils on egg hatching percentage of *Meloidogyne incognita*.

Sample	Concentration ($\mu\text{L}/\text{mL}$)	Percent egg hatching (mean \pm SD)			
		24 hr	48 hr	72 hr	96 hr
HCPNO	0.25	53.00 \pm 1.00 ^{fg}	61.00 \pm 1.00 ^d	68.33 \pm 2.08 ^c	76.33 \pm 0.57 ^b
	0.5	39.33 \pm 0.57 ^{lmn}	48.66 \pm 0.57 ^{hi}	54.00 \pm 1.00 ^{fg}	61.00 \pm 1.00 ^d
	1	15.00 \pm 1.00 ^{uvw}	18.00 \pm 1.00 ^{tuv}	25.33 \pm 0.57 ^{pq}	28.66 \pm 1.15 ^{op}
HCBO	0.25	47.00 \pm 1.00 ^{ij}	54.66 \pm 0.57 ^{efg}	61.66 \pm 0.57 ^d	76.00 \pm 2.00 ^b
	0.5	38.00 \pm 1.73 ^{mn}	41.66 \pm 0.57 ^{klm}	52.33 \pm 1.15 ^{fgh}	67.33 \pm 2.08 ^c
	1	13.00 \pm 1.00 ^w	14.33 \pm 1.52 ^{vw}	17.33 \pm 1.54 ^{tuv}	22.33 \pm 1.52 ^{qrs}
HCNO	0.25	58.33 \pm 0.57 ^{de}	69.00 \pm 1.00 ^c	78.66 \pm 0.57 ^{ab}	82.00 \pm 1.00 ^a
	0.5	42.33 \pm 0.57 ^{kl}	51.00 \pm 1.00 ^{gh}	55.66 \pm 0.57 ^{ef}	60.66 \pm 0.57 ^d
	1	17.1 \pm 1.00 ^{tuv}	19.33 \pm 0.57 ^{rst}	22.66 \pm 0.57 ^{qr}	28.66 \pm 1.15 ^{op}
HCPO	0.25	31.00 \pm 1.00 ^{lmn}	44.33 \pm 1.15 ^{jk}	45.00 \pm 1.73 ^{ijk}	56.00 \pm 1.73 ^{ef}
	0.5	30.66 \pm 0.57 ^o	36.66 \pm 0.57 ⁿ	40.33 \pm 0.57 ^{lmn}	38.33 \pm 0.57 ^{mn}
	1	12.66 \pm 1.15 ^w	15.33 \pm 0.57 ^{uvw}	18.66 \pm 1.15 ^{stu}	17.66 \pm 2.51 ^{tuv}
Control	Water	1.66 \pm 2.08	3.33 \pm 1.52	6.33 \pm 1.52	14.33 \pm 2.08

SD = standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh.

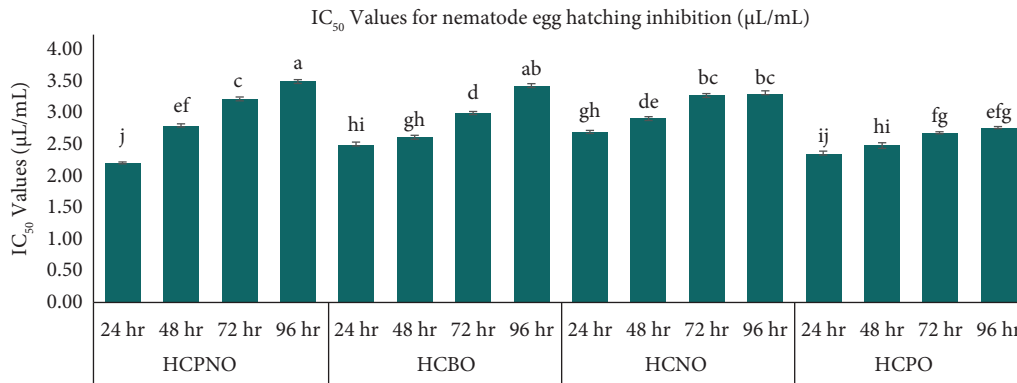


FIGURE 4: IC₅₀ values of essential oils on the egg hatching inhibition of *Meloidogyne incognita*. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh, error bar denoted the standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other).

and myrcene) might be responsible for the herbicidal activity of essential oil. Such compounds are also present in the composition of tested essential oils under study, which could be responsible for the herbicidal activity.

3.1.4. Antifungal Activity. Results showed that the essential oils exhibited significant antifungal activity in a dose-dependent manner by inhibiting the mycelial growth of pathogenic fungi (*Fusarium oxysporum* and *Curvularia lunata*). At higher concentrations (500 $\mu\text{L}/\text{mL}$), HCNO inhibited the mycelial growth by 86.29% and 84.81% of *F. oxysporum* and *C. lunata*, respectively, followed by HCPNO, HCBO, and HCPO. The antifungal activity of essential oils was considerably lower as compared to carbendazim (100.0%) (Table 11).

Joy et al. [10] reported a significant antifungal activity of *H. coronarium* rhizome essential oil against *Trichoderma* sp. and *Candida albicans*. In their study, 1, 8-cineole, β -pinene, and α -terpineol were found as major compounds of the essential oils that were supposed to be responsible for their

antifungal activity. Gullo et al. [22] evaluated the antifungal activity of *H. coccineum* rhizomes essential oil in which the major compounds were 1, 8-cineole and caryophyllene oxide that are also observed in the chemical composition of essential oils under investigation herein. Moreover, Ray et al. [19] reported the biologically active compounds (linalool, 1, 8-cineole, α -terpineol, terpin-4-ol, α -pinene, γ -terpinene, and β -pinene) of *H. coronarium* rhizome's essential oil effective against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum*. Similarly, another study has confirmed that the linalool, 1, 8-cineole, α -terpineol, terpin-4-ol, α -pinene, γ -terpinene, coronarin E, isopulegol, and (Z)-caryophyllene rich essential oil of *H. coronarium* were effective against *Alternaria*, *Fusarium*, and *Aspergillus flavus* [50]. Some of these compounds are also present in variable amounts in essential oils under present investigation. Therefore, it is inferred that the antifungal activity of the tested oils might be due to the presence of these biologically important compounds or due to the synergetic effect of other coexisting major or minor constituents of essential oils.

TABLE 6: Percent mortality of *S. litura*, treated with *H. coronarium* essential oils.

Essential oils	Concentration ($\mu\text{L}/\text{mL}$)	No. of insects used	No. of dead insects (mean \pm SD)			% of average mortality		
			24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
HCPNO	10	5	0.33 \pm 0.57 ^{gh}	0.66 \pm 0.57 ^{fgh}	1.00 \pm 1.00 ^{efgh}	6.66	13.00	20.00
	25	5	1.33 \pm 0.57 ^{defgh}	2.00 \pm 0.00 ^{b-h}	2.33 \pm 0.57 ^{B-G}	26.66	40.00	46.66
	50	5	2.33 \pm 0.57 ^{bcdefg}	2.66 \pm 0.57 ^{a-f}	3.00 \pm 1.00 ^{abcde}	46.66	53.33	60.00
	100	5	2.66 \pm 0.57 ^{a-f}	3.33 \pm 0.57 ^{abcd}	4.00 \pm 0.00 ^{ab}	53.33	66.66	80.00
HCBO	10	5	0.00 \pm 0.00 ^h	0.00 \pm 0.00 ^h	0.00 \pm 0.33 ^{gh}	0.00	0.00	6.66
	25	5	1.00 \pm 0.00 ^{efgh}	1.33 \pm 0.57 ^{defgh}	2.66 \pm 1.00 ^{b-h}	20.00	26.33	40.00
	50	5	1.33 \pm 0.57 ^{defgh}	2.66 \pm 1.57 ^{a-f}	3.00 \pm 0.00 ^{abcd}	26.66	53.33	66.66
	100	5	2.00 \pm 1.00 ^{b-h}	3.00 \pm 1.00 ^{abcde}	3.66 \pm 0.57 ^{ab}	40.00	60.00	80.33
HCNO	10	5	0.00 \pm 0.00 ^h	0.00 \pm 0.00 ^h	0.66 \pm 0.57 ^{fgh}	0.00	0.00	13.33
	25	5	0.66 \pm 0.57 ^{fgh}	1.33 \pm 0.57 ^{defgh}	1.66 \pm 0.57 ^{c-h}	13.33	26.66	33.33
	50	5	1.66 \pm 0.57 ^{cdefgh}	2.33 \pm 0.57 ^{bcdefg}	2.66 \pm 0.57 ^{a-f}	33.33	46.66	53.33
	100	5	2.00 \pm 1.00 ^{b-h}	3.00 \pm 1.00 ^{abcde}	3.66 \pm 0.57 ^{abc}	40.00	60.00	73.33
HCPO	10	5	0.00 \pm 0.00 ^h	0.00 \pm 0.00 ^h	0.00 \pm 0.00 ^h	0.00	0.00	0.00
	25	5	1.66 \pm 1.15 ^{c-h}	2.00 \pm 1.00 ^{b-h}	2.33 \pm 0.57 ^{bgg}	33.33	40.00	46.66
	50	5	2.33 \pm 0.57 ^{bcdefg}	3.33 \pm 0.57 ^{abcd}	2.66 \pm 0.57 ^{a-f}	46.66	66.66	53.33
	100	5	2.66 \pm 0.57 ^{a-f}	4.00 \pm 1.00 ^{ab}	4.66 \pm 0.57 ^a	53.33	80.00	93.33
Control	Water	5	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00	0.00	0.00

SD = Standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

TABLE 7: IC₅₀ values of *H. coronarium* essential oils for *S. litura* mortality.

Sample	Hr	IC ₅₀ (%) (mean \pm SD)
HCPNO	24	0.006 \pm 0.0012 ^b
	48	0.004 \pm 0.0008 ^b
	72	0.003 \pm 0.0009 ^b
HCBO	24	0.007 \pm 0.0026 ^b
	48	0.006 \pm 0.0024 ^b
	72	0.004 \pm 0.0016 ^b
HCNO	24	0.007 \pm 0.0021 ^b
	48	0.062 \pm 0.0012 ^a
	72	0.007 \pm 0.0014 ^b
HCPO	24	0.007 \pm 0.0022 ^b
	48	0.003 \pm 0.0009 ^b
	72	0.002 \pm 0.0014 ^b

HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh.

TABLE 8: Seed germination inhibition (in percent) by *H. coronarium* essential oils.

S. no.	Sample	% inhibition of seed germination (mean \pm SD)			
		50 $\mu\text{L}/\text{mL}$	100 $\mu\text{L}/\text{mL}$	150 $\mu\text{L}/\text{mL}$	200 $\mu\text{L}/\text{mL}$
1	HCPNO	36.00 \pm 2.00 ^b	51.33 \pm 0.57 ^c	78.00 \pm 1.00 ^g	92.33 \pm 2.08 ^h
2	HCBO	32.96 \pm 1.69 ^b	54.81 \pm 1.69 ^d	67.77 \pm 1.11 ^f	90.37 \pm 0.64 ^h
3	HCNO	21.85 \pm 1.69 ^a	56.29 \pm 0.64 ^{de}	73.33 \pm 1.11 ^g	90.00 \pm 1.11 ^h
4	HCPO	37.66 \pm 2.51 ^b	51.00 \pm 1.00 ^c	63.33 \pm 2.08 ^e	96.00 \pm 1.52 ^h
5	Pendimethalin*	100.00 \pm 0.00	100.00 \pm 0.00	100.00 \pm 0.00	100.00 \pm 0.00

*Standard herbicide; SD = standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

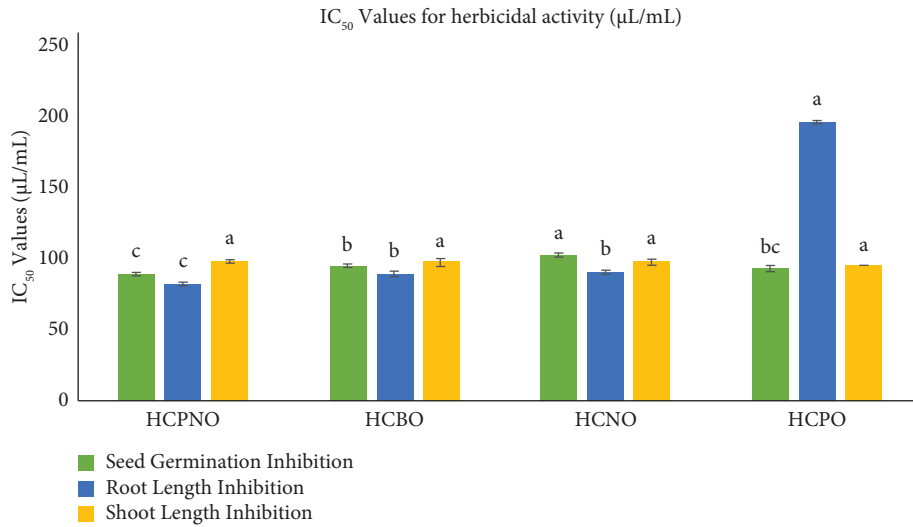


FIGURE 5: IC₅₀ values of *H. coronarium* essential oils for the herbicidal activity (seed germination inhibition, root length inhibition, shoot length inhibition) (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh, error bar denoted the standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other).

TABLE 9: Root length inhibition (in percent) by different *H. coronarium* essential oils.

S. no.	Sample	% inhibition of root length (mean ± SD)			
		50 μL/mL	100 μL/mL	150 μL/mL	200 μL/mL
1	HCPNO	24.81 ± 0.64 ^b	60.00 ± 2.22 ^f	84.44 ± 1.92 ^h	100.00 ± 0.00 ⁱ
2	HCBO	40.00 ± 1.11 ^d	46.66 ± 1.92 ^e	74.44 ± 1.11 ^g	99.62 ± 0.64 ⁱ
3	HCNO	33.33 ± 2.22 ^c	61.11 ± 1.11 ^f	75.18 ± 2.79 ^g	100.00 ± 0.00 ⁱ
4	HCPO	19.62 ± 0.64 ^a	27.77 ± 1.11 ^b	75.18 ± 2.79 ^g	99.62 ± 0.64 ⁱ
5	Pendimethalin*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

*Standard herbicide; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

TABLE 10: Shoot length inhibition by different *H. coronarium* essential oils.

S. no.	Sample	% inhibition of shoot length (mean ± SD)			
		50 μL/mL	100 μL/mL	150 μL/mL	200 μL/mL
1	HCPNO	31.66 ± 3.21 ^a	45.66 ± 1.15 ^c	77.66 ± 2.51 ^f	94.00 ± 1.00 ^h
2	HCBO	33.33 ± 1.52 ^{ab}	51.00 ± 1.00 ^d	62.33 ± 2.51 ^e	97.33 ± 0.57 ^{hi}
3	HCNO	31.00 ± 2.64 ^a	46.33 ± 1.52 ^{cd}	78.00 ± 1.00 ^f	93.66 ± 1.52 ^h
4	HCPO	38.51 ± 2.79 ^b	58.51 ± 1.69 ^e	84.44 ± 1.11 ^g	100.00 ± 0.00 ⁱ
5	Pendimethalin*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

*Standard herbicide; SD = standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

3.2. Correlation of Essential Oil Constituents and Biological Activities. Pearson's correlation coefficient of major essential oil compounds (>2.00%) and biological activities of tested essential oils suggested that there was no direct positive correlation of altitude with any chemical compound or biological activity. However, altitude was found to have moderate negative correlation with α -fenchene, thuja-2, 4(10)-diene, isoborneol, α -terpineol, myrtenal, 3, 4, 5, 5-tetramethyl-1, 3 cyclopentadiene carboxylic acid, α -terpinyl

acetate, longiborneol, and 5-ethyl-2-methyl-pyridin-4-amine. α -pinene, endo-fenchol, and terpinen-4-ol showed positive correlation with essential oil yield, whereas cis-limonene oxide was negatively correlated with oil yield. Among the chemical constituents of essential oils, caryophylla-4(12),8(13)-dien-5 α -ol, cis-dihydro mayurone, caryophyllene oxide, isobornyl acetate, camphene, (Z)-caryophyllene, and 3-octen-5-yne were positively correlated ($r \geq 0.8$) with nematode mortality. Nematode mortality was

TABLE 11: Effect of essential oils on percent mycelial growth inhibition of *F. oxysporum* and *C. lunata*.

	Concentration ($\mu\text{L}/\text{mL}$)	Percent mycelial growth inhibition (mean \pm SD)	
		<i>Fusarium oxysporum</i>	<i>Curvularia lunata</i>
HCPNO	50	27.40 \pm 0.64 ^b	28.51 \pm 0.47 ^{bc}
	100	33.70 \pm 0.64 ^c	41.85 \pm 2.79 ^d
	250	57.03 \pm 0.64 ^g	57.40 \pm 1.28 ^f
	500	84.11 \pm 1.25 ^j	83.30 \pm 0.06 ⁱ
HCBO	50	12.59 \pm 0.64 ^a	17.03 \pm 1.69 ^a
	100	28.14 \pm 0.64 ^b	30.37 \pm 0.64 ^c
	250	53.33 \pm 1.11 ^f	45.92 \pm 0.64 ^e
	500	80.77 \pm 0.67 ⁱ	78.96 \pm 0.92 ^h
HCNO	50	27.40 \pm 0.64 ^b	25.55 \pm 1.11 ^b
	100	47.44 \pm 0.72 ^e	38.59 \pm 0.71 ^d
	250	72.59 \pm 0.64 ^h	66.29 \pm 1.69 ^g
	500	86.29 \pm 0.64 ^j	84.81 \pm 1.28 ⁱ
HCPO	50	14.59 \pm 0.16 ^a	15.96 \pm 0.61 ^a
	100	38.88 \pm 1.01 ^d	26.66 \pm 1.11 ^{bc}
	250	57.77 \pm 1.11 ^g	57.40 \pm 1.28 ^f
	500	69.9 \pm 0.57 ^h	75.55 \pm 1.11 ^h
Carbendazim*	50	100.0 \pm 00	100.0 \pm 00
	100	100.0 \pm 00	100.0 \pm 00
	250	100.0 \pm 00	100.0 \pm 00
	500	100.0 \pm 00	100.0 \pm 00

* = Standard fungicide; SD = standard deviation; according to Tukey's test ($p < 0.05$), mean values in a column that are followed by the same letter do not substantially differ from each other. (HCPNO = *H. coronarium* essential oil from G.B.P.U.A. & T., Pantnagar, HCBO = *H. coronarium* essential oil from Chandika, Bageshwar, HCNO = *H. coronarium* essential oil from Dogaon, Nainital, HCPO = *H. coronarium* essential oil from MES colony, Pithoragarh).

negatively correlated with 1, 8, cineole, coronarin E, α -fenchene, thuja-2, 4(10)-diene, isoborneol, longiborneol, and 5-ethyl-2-methyl-pyridin-4-amine. Moreover, α -terpineol showed a significant positive correlation with nematode egg hatching inhibition, whereas, endo-fenchol was negatively correlated with this activity. Among the herbicidal activities, seed germination inhibition was positively correlated with α -pinene and endo-fenchol. Root length inhibition was positively correlated with α -terpineol and negatively correlated with isopulegol. Conversely, shoot length inhibition was in positive correlation with endo-fenchol and isopulegol and negatively correlated with α -terpineol. In terms of fungicidal activity, both the fungus, i.e., *Fusarium oxysporum* and *Curvularia lunata* showed positive correlation with α -terpineol and negative correlation with endo-fenchol. Moreover, *Fusarium oxysporum* also showed negative correlation with α -pinene whereas, *Curvularia lunata* was negatively correlated with isopulegol. In case of insecticidal activity, α -pinene endo-fenchol, and terpinen-4-ol were positively correlated whereas, α -terpineol was negatively correlated. The positive correlation suggests that the biological efficiency of the essential oils is possibly due to the presence of respective positively correlated compounds in the action of a single compound or synergistic action of more than one compound (Figure 6).

Among the chemical constituents of *H. coronarium*, α -pinene showed positive correlation of the constituents of HOEO except camphene, 3-octen-5-yne, linalool, cis-limonene oxide, α -terpineol, isobornyl acetate, α -terpinyl acetate, (*Z*)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, and caryophylla-4(12), 8(13)-dien-5 α -ol. α -fenchene showed

negative correlation with camphene, 3-octen-5-yne, linalool, endo-fenchol, *cis*-limonene oxide, isopulegol, isobornyl acetate, (*Z*)-caryophyllene, α -patchoulene, α -muurolene, caryophyllene oxide, *cis*-dihydromayurone, rosifoliol, and caryophylla-4(12), 8(13)-dien-5 α -ol. Camphene was in positive correlation with 3-octen-5-yne, isopulegol, terpinen-4-ol, isobornyl acetate, α -terpinyl acetate, (*Z*)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, and caryophylla-4(12), 8(13)-dien-5 α -ol. Thuja-2, 4(10)-diene was positively correlated with 1,8-cineole, isoborneol, terpinen-4-ol, α -terpineol, myrtenal, 3, 4, 5, 5-tetramethyl-1, 3 cyclopentadiene, carboxylic acid, α -terpinyl acetate, longiborneol, 5-ethyl-2-methyl-pyridin-4-amine, and coronarin E. 3-octen-5-yne was in negative correlation with the constituents of HOEO except isopulegol, terpinen-4-ol, isobornyl acetate, α -terpinyl acetate, (*Z*)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, and caryophylla-4(12), 8(13)-dien-5 α -ol. 1, 8-cineole showed negative correlation with *cis*-limonene oxide, isopulegol, isobornyl acetate, (*Z*)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, caryophylla-4(12), 8(13)-dien-5 α -ol. Linalool showed positive correlation with endo-fenchol, cis-limonene oxide, α -patchoulene, α -muurolene, rosifoliol, and coronarin E. endo-fenchol was in positive correlation with, isopulegol, terpinen-4-ol, α -patchoulene, α -muurolene, rosifoliol, and coronarin E. Similarly, *cis*-limonene oxide showed negative correlation with constituents of HOEO except α -terpineol, α -patchoulene, α -muurolene, rosifoliol, and coronarin E. Isopulegol was negatively correlated with HOEO constituents except terpinen-4-ol, isobornyl acetate, (*Z*)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, caryophylla-4(12), 8(13)-dien-5 α -ol. Isoborneol showed

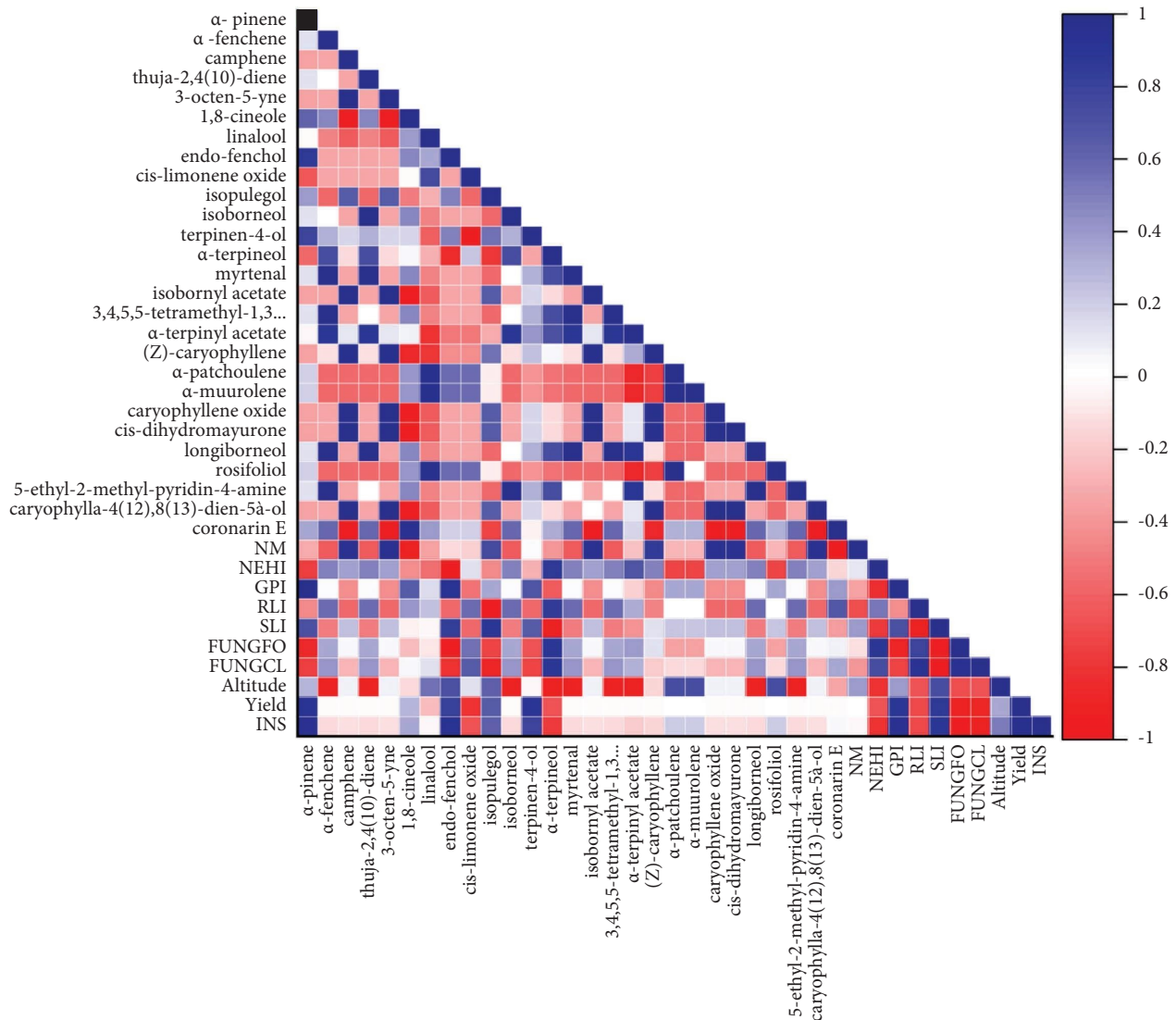


FIGURE 6: Correlation among major chemical compounds of *H. coronarium* essential oils and biological activities of essential oils. (Here, NM = nematode mortality, NEHI = nematode egg hatching inhibition; GPI = seed germination percent inhibition; RLI = root length inhibition; SLI = shoot length inhibition; INS = insecticide activity; FUNGFO = fungicide activity of *Fusarium oxysporium*; FUNGCL = fungicide activity of *Curvularia lunata*).

positive correlation with terpinen-4-ol, α -terpineol, myrtenal, 3, 4, 5, 5-tetramethyl-1, 3 cyclopentadiene, carboxylic acid, α -terpinyl acetate, longiborneol, 5-ethyl-2-methyl-pyridin-4-amine, coronarin E. terpinen-4-ol was negatively correlated with α -terpineol, α -patchoulene, α -murolene, and rosifoliol. Similarly, α -terpineol was positively correlated with isobornyl acetate, 3, 4, 5, 5-tetramethyl-1, 3 cyclopentadiene carboxylic acid, α -terpinyl acetate, (Z)-caryophyllene, longiborneol, 5-ethyl-2-methyl-pyridin-4-amine, and coronarin E. Myrtenal was positively correlated with 3, 4, 5, 5-tetramethyl-1, 3 cyclopentadiene, carboxylic acid, α -terpinyl acetate, longiborneol, 5-ethyl-2-methyl-pyridin-4-amine, and coronarin E. Isobornyl acetate showed positive correlation with α -terpinyl acetate, (Z)-caryophyllene, caryophyllene oxide, *cis*-dihydromayurone, caryophylla-4(12),8(13)-dien-5a-ol. Moreover, 3,4,5,5-tetramethyl-1,3 cyclopentadiene carboxylic acid was showed positive correlation with α -terpinyl acetate,

longiborneol, 5-ethyl-2-methyl-pyridin-4-amine, and coronarin E. α -terpinyl acetate was negatively correlated with α -patchoulene, α -murolene, and rosifoliol. Similarly, (Z)-caryophyllene was positively correlated with caryophyllene oxide, *cis*-dihydromayurone, and caryophylla-4(12),8(13)-dien-5a-ol. α -patchoulene had positive correlation with α -murolene, rosifoliol, and coronarin E. Whereas α -murolene had positive correlation with rosifoliol and coronarin E. Moreover, caryophyllene oxide was positively correlated with *cis*-dihydromayurone, and caryophylla-4(12), 8(13)-dien-5a-ol. *cis*-Dihydromayurone also positively correlate with caryophylla-4(12),8(13)-dien-5a-ol. Conversely, longiborneol was negatively correlated with rosifoliol, and caryophylla-4(12), 8(13)-dien-5a-ol. 5-ethyl-2-methyl-pyridin-4-amine and rosifoliol showed positive correlation with coronarin E whereas, caryophylla-4(12), 8(13)-dien-5a-ol was negatively correlated with coronarin E.

TABLE 12: *In silico* PASS prediction for antifungal, nematocidal, and insecticidal activities of selected phyto-compounds from different *H. coronarium* essential oils.

S. no.	Compound name	<i>Pass (Pa > Pi)</i>		
		Antifungal	Nematocidal	Insecticidal
1	1, 8-Cineole	0.214 > 0.128	0.191 > 0.070	0.159 > 0.058
2	(Z)-Caryophyllene	0.582 > 0.020	0.333 > 0.080	0.368 > 0.008
3	Linalool	0.596 > 0.019	0.372 > 0.021	0.436 > 0.005
4	Terpinen-4-ol	0.354 > 0.062	0.381 > 0.020	0.400 > 0.006
5	Coronararin E	0.471 > 0.036	—	0.112 > 0.105
6	Isopulegol	0.512 > 0.029	0.424 > 0.014	0.368 > 0.008
7	α -Terpineol	0.435 > 0.042	0.428 > 0.033	—
8	α -Terpinyl acetate	0.366 > 0.058	0.472 > 0.008	0.391 > 0.006
9	β -Atlantol	0.656 > 0.013	0.449 > 0.010	0.564 > 0.003
10	Rosifoliol	0.438 > 0.042	0.383 > 0.019	—
11	Caryophyllene oxide	0.349 > 0.063	0.215 > 0.077	0.112 > 0.104
12	Myrtenal	0.460 > 0.037	0.256 > 0.155	0.403 > 0.006

PASS = Prediction of activity spectra for substance; Pa = probable activity; Pi = probable inactivity.

TABLE 13: ADMET profile of major compounds of different *H. coronarium* essential oils.

Compounds	α -Pinene	1,8-Cineole (+)	Linalool	α -Terpineol	β -Caryophyllene	α -Terpinyl acetate	Coronararin E
TPSA* (\AA^2)	0.00	9.23	20.23	20.23	0.00	26.30	13.14
Consensus* log Po/w	3.44	2.67	2.66	2.53	4.24	3.04	5.22
Mol wt (g/mol)	136.23	154.25	154.25	154.25	204.35	196.29	284.44
nRB	0	0	4	1	0	3	2
nOHA	0	1	1	1	0	2	1
nOND	0	0	1	1	0	0	0
WLOGP	3.00	2.74	2.67	2.50	4.73	3.07	5.98
Water solubility	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Moderate
GI absorption**	Low	High	High	High	Low	High	Low
BBB permeant**	Yes	Yes	Yes	Yes	No	Yes	No
P-gp substrate**	No	No	No	No	No	No	No
CYP1A2 inhibitor**	No	No	No	No	No	No	No
CYP2C19 inhibitor**	No	No	No	No	Yes	No	Yes
CYP2C9 inhibitor**	Yes	No	No	No	Yes	Yes	Yes
CYP2D6 inhibitor**	No	No	No	No	No	No	No
CYP3A4 inhibitor**	No	No	No	No	No	No	No
Log K_p (cm/s) (skin permeation)	-3.95	-5.30	-5.13	-4.83	-4.44	-4.69	-3.64
Lipinski***	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lipinski violation	1	0	0	0	1	0	1
Bioavailability score***	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Hepatotoxicity****	No	No	No	No	No	Yes	No
Carcinogenicity****	No	No	No	No	No	No	No
Cytotoxicity****	No	No	No	No	No	No	No
Immunotoxicity****	No	No	No	No	Yes	No	Yes
Mutagenicity****	No	No	No	No	No	No	No
Predicted LD ₅₀ (mg/kg)****	3700	2480	2200	2830	5300	4800	2560
Toxicity class****	V	V	V	V	V	V	V

ADMET: absorption, distribution, metabolism, excretion and toxicity, lipophilicity*, pharmacokinetics**, drug likeness***, toxicological properties****, TPSA: topological polar surface area, nRB: no. of rotatable bonds, nOHA: no. of H-bond acceptor, nOND: no. of H-bond donor, WLOGP: water partition coefficient, GI absorption: gastrointestinal absorption, BBB: blood-brain barrier, P-gp: permeability glycoprotein, CYP: cytochrome P450, Toxicity class: (class I: fatal if swallowed ($LD_{50} \leq 5$), class II: fatal if swallowed ($5 < LD_{50} \leq 50$), class III: toxic if swallowed ($50 < LD_{50} \leq 300$), class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$), class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$), class VI: non-toxic ($LD_{50} > 5000$)).

3.3. *In Silico* PASS Prediction of the Major Compounds from Tested Essential Oils. Results of *In silico* PASS prediction advocated that, among all terpinen-4-ol, α -terpinyl acetate, β -atlantol, and rosifoliol compounds were found to possess significant Pa/Pi values for nematocidal activity. However, as predicted by PASS, several compounds were found to have very little nematocidal action (Table 12). These data are in

agreement with the *in vitro* nematocidal activity of the tested essential oils with compounds having significant Pa/Pi values. Among the selected compounds, (Z)-caryophyllene, linalool, coronararin E, isopulegol, and β -atlantol exhibited a good Pa/Pi range for the antifungal activity. However, some other compounds, such as 1,8-cineole, terpinen-4-ol, α -terpinyl acetate, and caryophyllene oxide were predicted

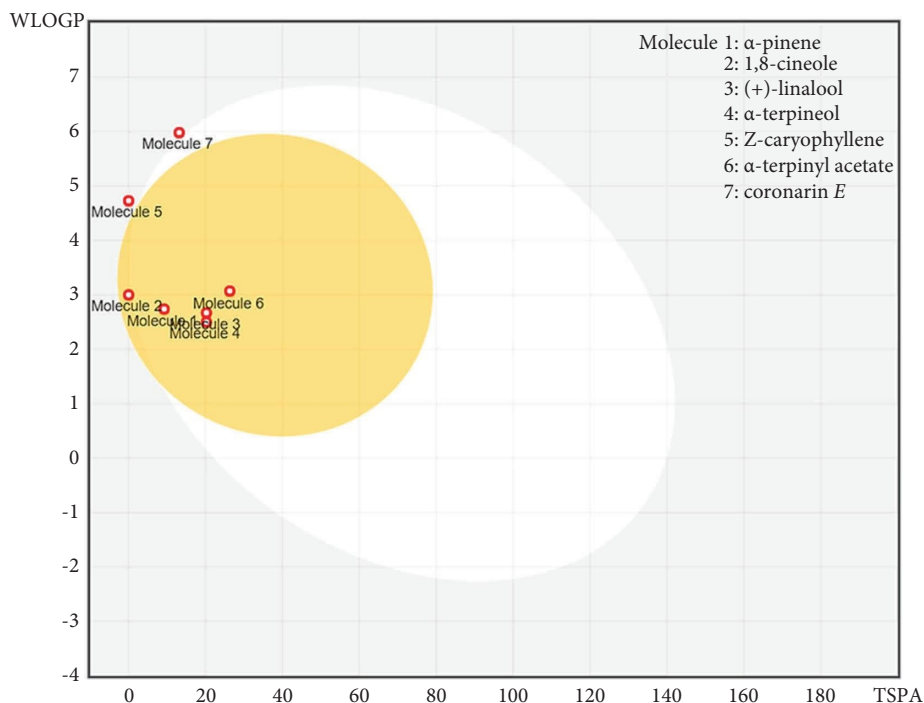


FIGURE 7: Boil egg prediction graph of the selected phytocompounds from different *H. coronarium* essential oils.

to have comparatively low antifungal activity in terms of $P_a > P_i$ values. Overall, the PASS prediction confirmed the correlation of compounds with *in vitro* antifungal activity. The P_a/P_i value of major compounds such as 1, 8-cineole, (Z)-caryophyllene, linalool, terpinen-4-ol, isopulegol, α -terpinyl acetate, β -atlantol, and myrtenal for the insecticidal potential is higher than those obtained for the antifungal activity. The PASS prediction reveals high insecticidal activities and moderate nematocidal and antifungal activity of the selected phytocompounds of tested essential oils.

3.4. ADMET Analysis. Considering that essential oils frequently fail to pass the antimicrobial/antibacterial drug testing because of their poor pharmacokinetics and metabolic performance [51, 52], we analyze the ADMET profile of some major constituents of the tested essential oils. All the ADMET properties of selected compounds were estimated and listed in Table 13. According to the crucial rules of drug-likeness, the compound should not violate more than 1 Lipinski rule, molecular weight should be less than 500 g/mol, topological surface area (TPSA) should be less than 140 \AA^2 , number of H-bond acceptors ($nOHA$) ≤ 5 , number of H-bond donors ($nOHD$) ≤ 5 , water partition coefficient (WLOGP) ≤ 5.88 , number of rotatable bonds (nRB) ≤ 10 [50, 51]. Based on the current findings all the compounds share TPAS less than 30 \AA^2 . High Gastrointestinal absorption (GI) was estimated for the compounds except for α -pinene, Z-caryophyllene, and coronarin E, thus the compounds can be easily absorbed by the gastrointestinal tract. Water solubility is also one of the important criteria to be drug effective, except for coronarin E all compounds were

found to be soluble in water. During the absorption processes, the first pass metabolisms via P-glycoprotein (P-gp) and cytochrome P450 enzymes in the small intestine and liver could negatively affect the bioavailability of drugs. According to the present findings, there was no P-glycoprotein (P-gp) substrate found, suggesting the good intestinal absorption of compounds while some of the compounds interacted mainly with 2 isoenzymes of the cytochrome (CYP450) family, namely CYP2C19 and CYP2C9 confirming their effectiveness with insignificant toxicity. The compounds that were predicted to not cross the blood-brain barrier (BBB) were Z-caryophyllene, and coronarin E as shown in the boil egg prediction graph (Figure 7). The compounds present in the yellow zone can permeate through the blood-brain barrier (BBB). Subsequently, the toxicological properties of the compounds were also estimated and listed in Table 13. None of the selected compounds possessed organ and oral toxic effects except α -terpinyl acetate (hepatotoxic), β -caryophyllene, and coronarin E (immunotoxic). From the results, it can be summarized that the compounds are suitable to be developed further as drug candidates.

4. Conclusion

In this study, the chemical diversity among the EOs of *H. coronarium* from different habitats and geographical locations of the Tarai and Kumaun regions of Uttarakhand was revealed and analyzed that varied in their respective yields (0.05 to 0.25%). GC-MS analysis revealed that all the essential oils were quite similar in their chemical profile and dominated by oxygenated monoterpenes. The most predominant compound present in the essential oils was 1,8-

cineole in varied quantities (18.2% to 12.0%). Based on *in vitro* biological assay it was found that *H. coronarium* EOs displayed significant nematocidal activity against *M. incognita* and very promising herbicidal activity against *R. raphanistrum*. Tested essential oils were found to possess moderate to good insecticidal activity against *Spodoptera litura*. The results revealed the potential properties of *H. coronarium* EOs as antifungal agents, herein, *C. lunata* was found to be more susceptible to essential oils than *F. oxysporum*. Therefore, *H. coronarium* EOs can be taken into account as a potential pest control agent against agricultural pests and diseases. However, the *in-vivo* studies and search for the bioactive compound responsible for the pesticidal properties from *H. coronarium* and exploration to identify the mechanism path for different pesticidal properties are further needed.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sushila Arya, Sonu Kumar Mahawer, and Himani Karakoti performed the experiment, analysed the data, and prepared the manuscript; Ravendra Kumar contributed in structuring the manuscript, supervision, review, and editing; Om Prakash contributed to experiment design for activity; Satya Kumar contributed to experiment design for activity; Mamta Latwal and Ganesh Pandey contributed in GC/MS analysis; Ravi Mohan Srivastava contributed to experiment design for activity; Mozaniel Santana de Oliveira contributed to review and editing; Dharmendra Singh Rawat contributed to plant material identification.

Acknowledgments

The authors acknowledge the G. B. Pant University of Agriculture and Technology, Pantnagar, India, for providing academic support and Central Instrumentation Center, University of Petroleum and Energy Studies (UPES), Bidholi campus, Dehradun, for providing facility for GC-MS analysis. The author, Dr. Mozaniel Santana de Oliveira, thanks PCI-MCTIC/MPEG, as well as CNPq for the scholarship (process number: (300983/2022-0)).

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