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Essential Oil Composition and *in vitro* Antifungal Activities of Essential Oil of Aerial Part of *Ocimum americanum* L. Collected from Kumaun Region of India

PANKAJ ARYA[1], MANOJ KARAKOTI[1], PRASOON KUMAR JOSHI[2,*] and RASHMI TEWARI[3]

[1]Department of Chemistry, M.B. Government P.G. College, Haldwani-263139, India
[2]Department of Chemistry, S.S.J.D.W.S.S.S. Government Post Graduate College, Ranikhet-263645, India
[3]Department of Plant Pathology, College of Agricultural, G.B. Pant University of Agriculture & Technology, Pantnagar-263145, India

*Corresponding author: E-mail: prasoonjoshi2012@gmail.com

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The essential oil of aerial part of *Ocimum americanum* L. was extracted by hydrodistillation process using Clevenger apparatus and analyzed by GC and GC-MS techniques. The yield of volatile oil was obtained as 0.27% (w/w) and a total 25 compounds were reported which constituted 96.28% of the total essential oil composition. Estragole is identified as major compound (69.54%) and linalool is reported as 5.28%. In the analysis of antifungal activities, the treatment appears to be most effective against *Fusarium oxysporum* and *Rhizoctonia solani*, with moderate effects on *Bipolaris maydis* and *Curvularia lunata*. *Sclerotinia sclerotiorum*, however, remains largely unaffected by the treatment across all concentrations, indicating that it may require different or higher concentrations for effective control. The essential oil of *Ocimum americanum* exhibits the strongest antifungal activity against *F. oxysporum*, with the lowest IC_{50} value of 95.3675 ppm. This suggests that *F. oxysporum* is the most susceptible to the essential oil, requiring the least concentration to inhibit 50% of fungal growth. In contrast, the essential oil shows the weakest activity against *S. sclerotiorum*, with an IC_{50} value of 634.294 ppm, indicating that it requires a higher concentration to achieve the same level of inhibition.

Keywords: *Ocimum americanum,* Essential oil composition, Estragole, Antifungal activities.

INTRODUCTION

Since acient times, *Ocimum* extract is used for the common cold, head aches, stomach aches, nervousness, heart illness as well as in the treatment of malaria [1-5]. *Ocimum* belongs to the Lamiaceae family are very important for their therapeutic potential. The genus *Ocimum*, also known as basil, has long been known for source of essential oils, its flavour, spice as well as its aroma [6] and includes more than 150 species and is known as one of the largest genera of the Lamiaceae family [7]. Basil leaf paste was also used for the treatment of skin diseases [8] and some volatile compounds those are obtained from *Ocimum* have been reported as allelopathic agents [6]. The leaf juice of *O. americanum* is used to manage dysentery, toothache in traditional medicine and also the leaf has been documented to be used in treatment of malarial fever and nasal bleeding [9-13]. *O. americanum* essential oil has been reported for its antibacterial activity against *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhos* and *Streptomycespyo genes* [14]. The essential oil of *O. americanum* is also reported for its antifungal activity against a large number of fungi as well as against some human pathogens [15].

Estragole, a volatile terpenoid, is naturally found in various herbs and spices. It is commonly used in food additives, soaps and detergents due to its essential oils [16]. According to the FDA, its safe concentration range is less than 1.9242 mg/kg of body weight per day. Studies have shown that extracts of *Pimenta racemosa*, which contain estragole, exhibit potent antibacterial properties that can inhibit *Pseudomonas syringae* and help control bacterial canker disease in kiwi fruit [17-19]. The present study deals with the antifungal activity of essential oil of the aerial part of plant species against five soil born fungal pathogens *F. oxysporum*, *S. sclerotiorum*, *B. maydis*, *C. lunata* and *R. solani* as well as the chemical composition of essential oil of *O. americanum*. The *in vitro* antifungal activity of the

essential oil was tested by the poisoned food technique using Potato- dextrose-agar (PDA) medium against the five test fungi. This report presents the essential oil composition of *Ocimum americanum*, collected from the Kumaun region of Uttarakhand, India. Significantly, estragole is identified as the most prevalent compound. Furthermore, previous literature indicates a lack of reports utilizing the poisoned food technique to evaluate these essential oils against the five selected fungal pathogens.

EXPERIMENTAL

All the chemicals were of AR Grade supplied by E. Merck (India), Thomas Baker (Mumbai) and S.D. Fine Chemicals.

Identification of plant species: Aerial part of the fresh plant (*O. americanum*) was collected from Haldwani town of Kumaon region of Uttarakhand (29.2150649ºN,79.5043602ºE) at an elevation of about 424 m from sea level during the month of November 2021. Identification of plant was done at Regional Ayurveda Research Institute under Central Council for Research in Ayurvedic Sciences (Ministry of AYUSH, Government of India) Ranikhet, India (voucher with specimen no. 10 and accession no. 2402).

Extraction of essential oil: The aerial part of *O. americanum* thoroughly washed with tap water, after that a Clevenger apparatus was used for hydrodistillation with 2.5 L of water for 3 h. The distillation was done in triplicate. The obtained volatile oil was dried over anhydrous sodium sulphate and then stored in a sealed vials in refrigerator.

Preparation of stock solution: The stock solution of desired concentration of essential oil *viz.* 10,000 ppm was prepared by adding requisite amount of essential oil, sterile distilled water, 10% DMSO and 1% Tween-20 (used as an emulsifying agent for the preparation of stable oil-in-water emulsions). This stock solution is then added in different volume into 60 mL PDA [20] for triplicate for each treatment of concentration for assigning bioassay of essential oil against different fungal pathogen [21].

***In vitro* antifungal activity:** The antifungal activity of essential oil had been tested by the poisoned food technique [22] using potato-dextrose-agar (PDA) medium. The soil-borne pathogenic fungi *viz. F. oxysporum, S. sclerotiorum*, *B. maydis*, *C. lunata* and *R. solani* were obtained from the Department of Plant Pathology, Oil Seed Lab, College of Agriculture, GBPUA &T, Pantnagar, India. These fungal cultures were maintained and revived on potato dextrose agar (PDA) and stored at > 4 ºC. Then 20 mL of sterilized medium were poured into sterilized Petri plates. A 5 mm disc of fungal growth from a 10-days old culture on PDA was cut using a cork borer for each fungal species. The discs were then placed onto sterilized PDA plates in such a way that the fungal growth came into contact with the PDA. The plates were incubated for 7 days at 25 ± 2 ºC. After incubation, the radial growth was measured.

Bioassay of essential oils against different pathogen

Poisoned food technique: In the freshly prepared potato dextrose agar medium, added required amount of essential oil as to get a final desired concentration and thoroughly mixed. The fungi culture was multiplied growing on PDA medium for 7 days at 25 ± 2 ºC. Small disc of fungus culture was cut with sterile cork borer and transferred aseptically in the centre of the Petri-dish containing the medium having desired essential oil concentration. Suitable evaluations using the culture discs on PDA without essential oil were conducted. The control sets were prepared by using equal amount of 10% DMSO in place of oil. The plates were incubated at 25 ± 2 ºC in BOD incubator. The fungal colony diameter is measured at every 24 h. The colony diameter, measured at each concentration of essential oil is compared with check to evaluate the toxicity of essential oil towards the test fungus.

For each essential oil, the different concentration of respective essential oil was prepared by dissolving weighed quantity of essential oil in a measured volume of sterilized distilled water. The amount of solution to be added to PDA medium was calculated by the following formula:

$$C_1V_1 = C_2V_2$$

where C_1 = concentrations of stock solution (µg/mL); C_2 = desired concentration (µg/mL); V_1 = volume of stock solution to be added; V_2 = measured volume of PDA medium.

The required amount of each essential oil was added to achieve concentrations of 25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm, with thorough mixing before plating. Then, 20 mL of culture medium, with different treatments, were poured into each Petri plate inoculated with the treated fungi. A 5 mm mycelial disc from a 10 days old culture of each fungal isolate was inoculated separately and incubated at 25 ± 2 ºC for 7 days. The radial growth was measured in millimeters using a scale. The percentage inhibition was calculated using the following formula [23]:

$$\text{Inhibition } (\%) = \frac{X - Y}{X} \times 100$$

where X = radial growth in check, Y = radial growth in treatment.

Statistical analysis: The data were analyzed statistically at the computer centre of G.B. Pant University of Agriculture and Technology, Pantnagar, India using complete randomized block design (CRD) as per the requirement of the experiment. Treatments were compared by mean of critical differences at 95% level of significance and F-Stat wherever required.

GC & GC-MS conditions: The GC-MS analysis of the volatile oil was done by GC-MS Shimadzu QP-2010 Ultra GC, which was fitted with Rxi-5 Sil MS consisting of 30 m in length and 0.25 mm in diameter with film thickness of 0.25 µm. The oil sample was diluted using acetone as a solvent. Helium was used as carrier gas with constant flow rate of 1.21 mL/min and average velocity of 39.9 cm/s. The pressure was kept 69.0 kPa and the temperature of essential column oven was programmed from 50 ºC with hold for 2 min to 210 ºC with hold for 2 min at the rate of 3 ºC/min to the final temperature of 280 ºC with hold for 13 min at the rate of 6 ºC/min. The temperature of injector was kept at 260 ºC with split ratio of 1:10. The detector was used as flame ionization detector. The mass spectrometry analysis was done at 70 eV with mass scan range of 45-650 amu. The temperature of ion source was 230 ºC and the temperature of interface was 280 ºC.

The compounds present in the volatile oil of *O. americanum* were identified on the basis of their retention indices (RI) data, which was determined concerning a homologous series of *n*-alkane (C_8-C_{33}) indices on Rtx-5MS capillary column of fused silica. The identification of the compounds was based on MS Library search as well as by comparing it with the literature MS data and their published RI values [24-27]. The relative percentage of volatile oil constituents was in their respective percentage by peak area normalization (FID response) without using the correction factor.

RESULTS AND DISCUSSION

The gas chromatogram of the essential oil obtained from the aerial part of *O. americanum* is shown in Fig. 1. The yield of oil was found as 0.27% w/w (1.53 g) and the identified compounds of oil are listed in Table-1. Total 27 compounds were identified accounting 97.11% of total composition of volatile oil. The composition analysis of the volatile oil reveals that monoterpenoids (84.33%) and sesquiterpenoids (12.78%) are present majorly.

In previous report [28], estragole is found only 1.7% in the volatile oil of *O. americanum* collected from the Kumaun Hills of Nainital, India. The authors were also reported that estragole was found as 10.2% in volatile oil of *O. americanum* collected from Banbasa, 11.7% collected from Rushi, 12.9% collected from Champawat, 1.3% collected from Kilbury and 28.9% collected from Dhoulchina [28]. Estragole was totally absent in the volatile oil of *O. americanum* collected from Rudrapur and Almora [28], while estragole is present as 69.54% in the present study and found as the major compound in essential oil of *O. americanum* collected from Haldwani region of Kumaun Hills. The second major compound was linalool (5.28%), which was found in trace amounts in the volatile oils of *O. americanum* from Champawat, Rudrapur and Nainital and absent in Banbasa and Rashi, 14.2% in Dharchula, 2.2% in Almora and 3.0% in Kilbury [28]. The differences in oil yield and chemical composition, when compared to previous studies, could be attributed to genetic factors, environmental conditions, harvesting techniques and the method of oil extraction [29-31]. Estragole (69.54%) as major compound is reported for the first time from Kumaon region of Uttrakhand state for this plant. Thus, the volatile oil of *O. americanum* collected from Haldwani region of Kumaun, Uttarakhand, India is estragole rich.

Antifungal activity: The antifungal activities data presented in Table-2 provides the growth inhibition of five fungal species, namely *F. oxysporum*, *S. sclerotiorum*, *B. maydis*, *C.*

TABLE-1
ESSENTIAL OIL COMPOSITIONS OF AERIAL PART OF *O. americanum* L. COLLECTED FROM KUMAON REGION OF UTTARAKHAND, INDIA

S. No.	Compound	Retention time	RI (Obsd.)	RI [24-27] (Lit)	% Composition
1	α-Pinene	6.527	929	933	0.12
2	Camphene	7.073	945	943	0.11
3	β-Pinene	8.063	974	978	0.15
4	Myrcene	8.613	990	991	1.10
5	3-Hexenol acetate	9.357	1009	992	0.05
6	Eucalyptol	10.257	1031	1035	1.91
7	E-β-Ocimene	11.010	1049	1046	3.37
8	Terpinolene	12.547	1085	1086	0.18
9	Linalool	13.603	1109	1099	5.28
10	Camphor	15.353	1142	1149	2.11
11	Lavandulol	16.780	1179	1172	0.35
12	Estragole	19.063	1195	1201	69.54
13	Bornyl acetate	21.607	1286	1285	0.06
14	Copaene	25.353	1372	1375	0.05
15	β-Elemene	26.080	1389	1390	1.65
16	E-Caryophyllene	27.197	1432	1424	1.24
17	*cis*-Muurola-4(14),5-diene	28.993	1459	1466	0.26
18	Germacrene D	29.770	1478	1480	0.44
19	Bicyclogermacrene	30.383	1493	1497	1.33
20	Bulnesene	30.650	1499	1505	0.84
21	γ-Cadinene	31.160	1512	1512	1.24
22	E-Nerolidol	33.333	1568	1566	0.09
23	Spathulenol	33.810	1580	1576	0.21
24	Di-1,10-epicubenol	35.210	1616	1618	0.56
25	epi-α-Cadinol	36.450	1649	1640	3.70

Class of compounds identified total %

S. No.	Class of compounds identified	S. No.	Total %
1	Monoterpene hydrocarbons	1-4,7	4.85
2	Monoterpene oxygenated	5,6,8-13	79.48
3	Sesquiterpene hydrocarbon	14-21	7.39
4	Sesquiterpene oxygenated	22-25	4.56
	Total % identified		96.28

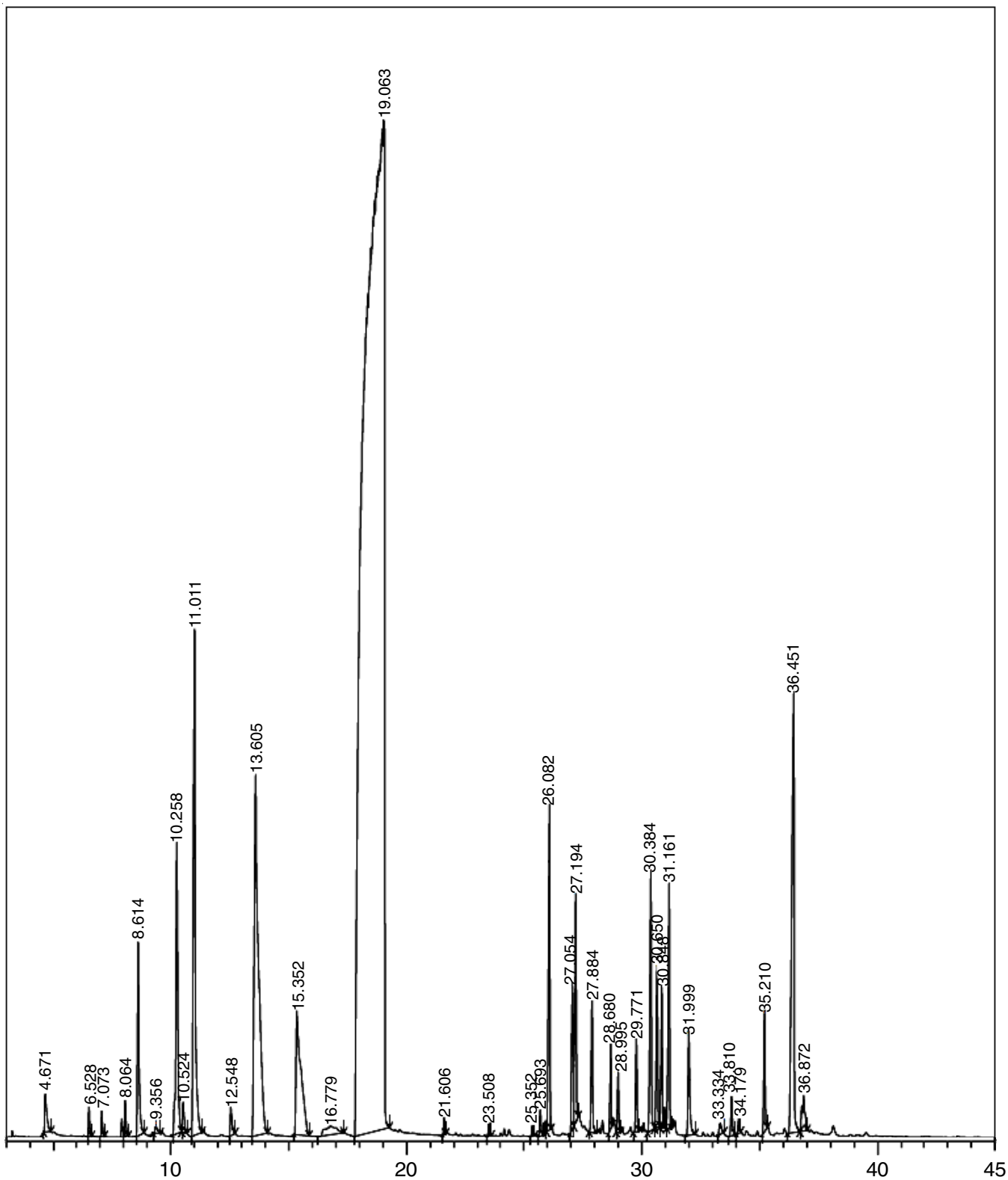

Fig. 1. GC-MS spectrum of volatile oil of aerial part of *O. americanum* L.

lunata and *R. solani* under varying concentrations of a certain treatment (25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm), with the control group showing no inhibition. At control (0 ppm), all fungal species showed no inhibition of growth, with an average growth value of 85 mm for all species. At 25 ppm, *F. oxysporum* exhibited the highest inhibition (28.33 mm), followed by *B. maydis* (14.34 mm), *C. lunata* (20.66 mm) and *R. solani* (14.33 mm). Interestingly, *S. sclerotiorum* showed no inhibition at this concentration, maintaining the same growth as the control group (85 mm). At 50 ppm, the inhibition effect intensified as *F. oxysporum* (39.34 mm), *B. maydis* (33.66 mm) and *C. lunata* (24.33 mm), all showed the significant growth

TABLE-2
ANTIFUNGAL ACTIVITIES OF ESSENTIAL OIL OF AERIAL PART OF *O. americium* AGAINST FIVE DIFFERENT FUNGI

Concentration		*Fusarium oxysporum*	*Sclerotinia sclerotiorum*	*Bipolarish maydis*	*Curvularia lunata*	*Rhizoctonia solani*
Control	Growth	85.00 ± 0.00	85.00 ± 0.00	85.00 ± 0.00	85.00 ± 0.00	85.00 ± 0.00
	Inhibition (mm)	0	0	0	0	0
25 ppm	Growth	56.66 ± 0.53	85.00 ± 0.00	70.66 ± 0.53	64.66 ± 0.53	70.66 ± 0.53
	Inhibition (mm)	28.33	0	14.34	20.66	14.33
50 ppm	Growth	45.66 ± 0.53	85.00 ± 0.00	51.33 ± 1.41	60.66 ± 0.53	62.00 ± 1.85
	Inhibition (mm)	39.34	0	33.66	24.33	23.00
100 ppm	Growth	36.66 ± 1.41	81.33 ± 0.53	47.33 ± 0.53	52.00 ± 0.92	42.33 ± 1.92
	Inhibition (mm)	48.34	3.67	37.67	33.00	42.66
250 ppm	Growth	23.33 ± 1.41	76.66 ± 1.41	40.33 ± 0.53	44.66 ± 1.07	25.66 ± 1.41
	Inhibition (mm)	61.67	8.34	44.66	40.33	59.33
500 ppm	Growth	16.00 ± 0.92	49.66 ± 1.41	33.33 ± 1.92	24.33 ± 1.41	0.00 ± 0.00
	Inhibition (mm)	69.00	35.34	51.66	60.66	85.00
ANOVA	F-Stat	0.00008923	0.01071	0.003061	0.004132	0.002767
	P value	0.9999	0.9894	0.9969	0.9959	0.9972

At significant level 95%

TABLE-3
IC_{50} VALUE OF ESSENTIAL OIL OF *O. americium* AGAINST FIVE SELECTED FUNGI

	Fusarium oxysporum	*Sclerotinia sclerotiorum*	*Bipolarish maydis*	*Curvularia lunata*	*Rhizoctonia solani*
Conc. (ppm)	95.3675	634.294	288.06	269.034	166.817

reductions. *S. sclerotiorum* still showed no inhibition at this concentration whereas *R. solani* exhibited an inhibition of 23 mm. At higher concentration 100 and 250 ppm, the growth inhibition increased further for most species (Table-2). At 500 ppm, the maximum inhibition was observed, especially for *R. solani*, which showed total growth inhibition (85 mm). *F. oxysporum* and *C. lunata* had significant inhibition of 69.00 mm and 60.66 mm, respectively. *B. maydis* showed an inhibition of 51.66 mm, whereas *S. sclerotiorum* showed a moderate inhibition of 35.34 mm, although not as substantial as the other species.

The F-statistic and p-values presented for each fungus are important for understanding whether the observed differences in growth are statistically significant. The *p*-values for *F. oxysporum* (0.00008923), *B. maydis* (0.01071), *C. lunata* (0.003061) and *R. solani* (0.002767) indicate statistically significant differences in the growth inhibition across different concentrations. A p-value < 0.05 suggests that the treatment had a significant effect on growth inhibition for these fungi. However, *S. sclerotiorum* had a *p*-value of 0.9999, which is well above 0.05, indicating that the treatment did not significantly affect its growth across all concentrations tested. The essential oil of *O. americanum* exhibits the strongest antifungal activity against *F. oxysporum*, with the lowest IC_{50} value of 95.3675 ppm. This suggests that *F. oxysporum* is the most susceptible to the essential oil, requiring the least concentration to inhibit 50% of fungal growth. The IC_{50} values of essential oil of *O. americium* against five selected fungi are represented in Table-3. In contrast, the essential oil shows the weakest activity against *S. sclerotiorum*, with an IC_{50} value of 634.294 ppm, indicating that it requires a higher concentration to achieve the same level of inhibition. *B. maydis* (IC_{50} = 288.06 ppm), *C. lunata* (IC_{50} = 269.034 ppm) and *R. solani* (IC_{50} = 166.817 ppm) fall in the intermediate range. These fungi are not as susceptible as *F. oxysporum*, but they are more sensitive than *S. sclerotiorum.*

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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