Journal of Applied Biological Sciences
E-ISSN: 2146-0108 14(3): 296-308, 2020
Research Article

CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *RHIZOPOGON* SPECIES AGAINST FUNGAL ROT OF APPLE

Mehrajud Din Talie[1], Abdul Hamid Wani[1], Bashir Ahmad Lone[2], Mohd Yaqub Bhat[1]*

[1]*Section of Mycology and Plant Pathology, Department of Botany University of Kashmir, Srinagar 190006 J&K, India*
[2]*Natural Product Chemistry Division, CSIR, Indian Institute of Integrative Medicine (IIIM) Jammu (180001)*

**Corresponding author:*
E-mail address: myaqub35@gmail.com

(Received 09th May 2020; accepted 22th October 2020)

ABSTRACT. Gas chromatography-mass spectrometry (GC-MS) is one of the so-called hyphenated analytical technique. GC-MS profiling of n-Hexane extract of the ecologically important mushroom *Rhizopogon* species was investigated for first time from Kashmir Valley and 37 volatile and semi-volatile organic compounds were detected. Among them various major bioactive compounds viz. 1-tetradecene, 2, 4-di-tert-butylphenol, Cetene, 1-octadecene, n-Hexadecanoic acid, 1-nonadecene, cis-vaccenic acid, Behenic alcohol and n-tetracosanol are believed to be responsible for their strong antifungal activity against all the test fungal pathogens. Therefore, different concentrations of n-Hexane extract of *Rhizopogon* species were evaluated for their antifungal activity against fungi such as *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata* causing postharvest disease of apple. It was revealed from the present investigation that all the concentrations exhibited significant antimycotic activity against all the test fungal pathogens. However, maximum spore germination inhibition was observed in *Penicillium chrysogenum* (87.80%) followed by *Aspergillus niger* (71.08%) and *Alternaria alternata* (55.10%) respectively, at highest concentrations (200 mg/ml) of *Rhizopogan* species extract. Similarly, the maximum zone of inhibition was shown in *Penicillium chrysogenum* (27.00 ± 1.00) followed by *Aspergillus niger* (22.67 ± 2.08) and *Alternaria alternata* (18.57 ± 1.52) respectively, at highest concentrations (200 mg/ml). Other concentrations (100 mg/ml and 150 mg/ml) of n-Hexane extract also caused spore germination inhibition and zone of inhibition of the test fungi but to a lesser extent.

Keywords: *antifungal activity, GC-MS profiling, Mushrooms, organic compounds, Rhizopogon species*

INTRODUCTION

Mushrooms have long association with humankind and provide profound biological and economic impact. From times immemorial, wild macrofungi are consumed by humans with delicacy, due to their sweet taste, aroma and pleasing flavour [1]. They are believed to be rich source of nutrients with high content of proteins, minerals, fibers, trace elements, vitamins and cholesterol [2, 3]. Various macrofungi have been used in folk medicine for thousands of years due to the presence of various nutraceuticals [4, 5]. Macrofungi are well-known as rich source of numerous bioactive compounds which have antifungal, antiviral, antibacterial, antiparasitic, antioxidant, antiinflammatory, antiproliferative, anticancer, antitumour, cytotoxic, anti-HIV, hypo-cholesterolemic, antidiabetic, anticoagulant and hepatoprotective properties [6, 7, 8].

The *Rhizopogon* genus contains approximately 150 species of hypogeous or subepigeous ectomycorrhizal fungi [9]. They usually show ectomycorhizal association with the members of Pinaceae and are distributed throughout the world in natural habitats [10]. *Rhizopogan* genus plays an important ecological role in the establishment, growth and maintenance of these natural habitats [11, 12].

Gas chromatography-mass spectrometry (GC-MS) is a very powerful and ubiquitous analytical technique for the identification and quantification of unknown volatile and semi-volatile organic substances in complex mixtures by determining their molecular weights and their elemental composition [13]. The metabolite profiling of biological materials based on GC-MS analysis is one of the key technologies that significantly contributes to the understanding of the metabolome. Therefore, GC-MS based metabolite profiling is now used as ideal technology for screening of important phenotypic characters in functional genomic investigations of plants and microbes that include bacteria and fungi [14, 15].

Malus domestica (Borkh.) is most important temperate fruit crop, cultivated in Asia and Europe since times immemorial [16]. The Kashmir Valley is the leading producer of apple (*Malus domestica* Borkh.) in India. About 77% of apple production in India comes from Kashmir and its share to India's total production is increasing [17]. Apple rot is an economically significant disease on apple caused by several fungi like *Penicillium* species, *Alternaria alternata*, *Fusarium* species, *Botrytis cinerea*, *Mucor* species, *Rhizopus stolonifer* etc. that are common and causing significant economic losses in USA, Europe and other parts of the world [18, 19, 20]. Postharvest disease of fruits and vegetables accounts for about 10-30% loss of food production mostly in developing countries such as India, especially in Kashmir Valley [21].

Keeping in view, the huge economic losses to apple industry and farmers in Kashmir Valley due to the postharvest disease of apple caused by *Penicillium chrysogenum* Thom (1910), *Aspergillus niger* Van Tieghem (1867) and *Alternaria alternata* (Fr.) Keissl. (1912). The present study was investigated for the first time from Kashmir Valley for identification and quantification of unknown volatile and semi-volatile organic substances in complex mixtures from n-Hexane extract and screened for their antifungal activity against fungi causing rotting of apple in Kashmir Valley.

MATERIALS AND METHODS

Sample collection and identification

The mushroom specimen needed for the present research work were collected from Wadpora area of Kupwara district of Northern Kashmir (Fig.1). Identification of the collected mushroom was done based on morphological, reproductive features and analysis of ITS sequences.

Fig. 1. *Fruiting bodies of Rhizopogon species*

Low-pressure extraction (LPE)

The fruiting bodies were first washed with distilled water thoroughly to free them from mud and other extraneous materials, placed on blotting paper, chopped into small pieces and dried at 40ºC for 24 hours. The dried fruiting body samples were ground into fine powder with grinder and sealed in airtight plastic bags for storage purpose, prior to extraction.

Soxhlet extraction was performed according to the method of Association of Analytical Communities [22]. The n-Hexane was used as a solvent for Soxhlet extraction procedure in the ratio of 1:4 for 12 hours at its boiling temperature. The extract was then evaporated to complete dryness under reduced pressure using rota evaporator at 40ºC temperature and stored for further use. From hexane extract we got volatile oil having essence and about 1mg of same dried extract was dissolved in 1ml of HPLC grade Ethyl acetate and subjected to GC-MS analysis.

GC-MS Analysis

Gas chromatograph

Perkin Elmer (USA) make gas chromatograph model auto system XL was used to analyse the chemical constituents of n-Hexane extract. The GC was equipped with flame ionization detector (FID) and Restek (Bellefonte, PA, USA) make fused silica capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) coated with dimethyl polysiloxane (RTX-1).

Gas chromatograph-mass spectrometer

GC-MS analysis was carried out on a Varian GC MS 4000 fitted with a Varian Factor Four VF-5 MS fused silica capillary column (30 m × 0.25 mm id, film thickness 0.25 um).

GC analysis

Column oven temperature was automated from 50-240ºC at 5ºC per min. Injector as well as detector temperatures were adjusted at 250ºC and 270ºC respectively. Nitrogen gas was prefferd as the mobile phase during the present study at a flow rate of 1mL/min with injector split ratio of 1:80.

GC-MS analysis

Temperature setup of oven was maintained from 50 to 240ºC at 5ºC/min rising rate. The carrier gas used during the present investigation was Helium at flow rate of 1 mL/min. Mass spectra were documented over 50-300 amu range at 1 scan per sec with E.I. at 70 eV.

Identification of derived peaks was done by comparing their retention times with reference samples injected under similar chromatographic conditions. The mass spectra were compared with those reported in the NIST and WILEY computer libraries and those published in literature.

Collection of fruit samples

The infected fruit samples of apple were collected from markets, godown, cold storage and storage houses of Kashmir Valley (Fig. 2). These were packed in air tight polythene bags and brought to Plant Pathology and Mycology Laboratory, Department of Botany, University of Kashmir, Srinagar (190006) for pathological studies.

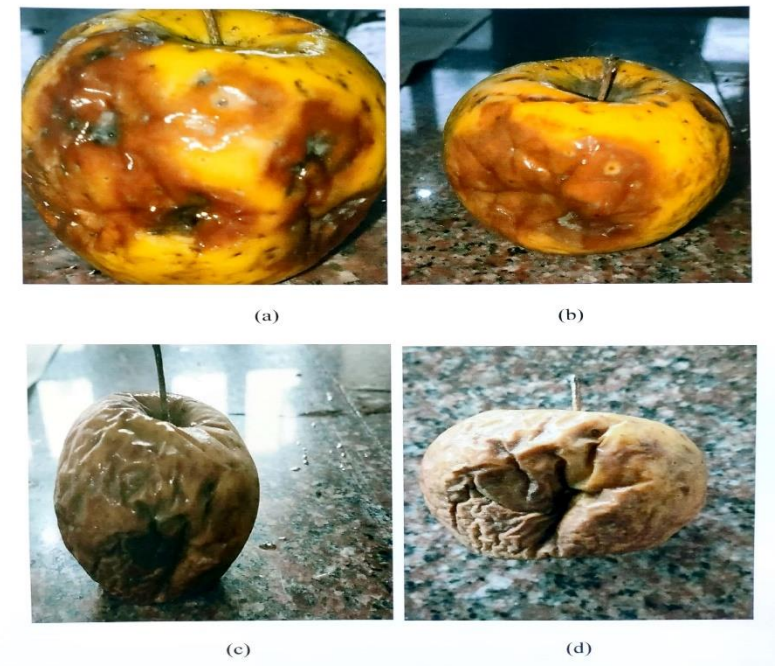

Fig. 2. *Apple Samples infected by different fungi.*

Isolation of fungi from apple

Sterilization of diseased samples of apple was done in 1% sodium hypochlorite solution followed by washing with sterilized double distilled water. Small sterilized samples were then inoculated on Petr plates containing Potato Dextrose Agar (PDA) medium and were incubated at 25±2ºC for 5 to 8 days for proper growth. Individual colonies from mixed or mother culture were sub-cultured on fresh petri plates containing PDA medium for pure culture of each isolate. The pure culture was maintained for proper identification of rot causing fungi.

Identification of fungi

The isolated fungi were identified on the basis of their morphological, reproductive, and cultural characteristics [23, 24]. The lectophenol cotton blue stain was used to prepare fungal slides for identification purpose. These slides were observed under Trinocular microscope and further identification was done by consulting different monographs and relevant literature.

Antifungal assay

The antifungal activity of n-Hexane extract of *Rhizopogon species* was assessed by making different concentrations.

Test organisms

The test fungal organisms used during the present study such as *Penicillium chrysogenum* Thom (1910), *Aspergillus niger* Van Tieghem (1867) and *Alternaria alternata* (Fr.) Keissl. (1912) were isolated and identified from diseased apple samples in Section of Mycology and Plant Pathology, Department of Botany University of Kashmir, Srinagar 190006 J&K, India.

Spore germination assay

To evaluate the efficacy of different concentrations of n-Hexane extract of *Rhizopogon* species on spore germination of test fungi, different concentrations, viz. 100 mg/ml, 150 mg/ml and 200 mg/ml were prepared from the precipitated sample. Spore suspension with 1×10^3 conidia/ml was prepared in sterilized distilled water. Equal volumes of both mushroom extract as well as spore suspension were mixed in a sterilized test tube and then shaken vigorously. The mixture then contained the specific concentration of test n-Hexane extract. Similarly, for control sucrose solution was mixed with equal volume of spore suspension. Now, from the mixture about 0.1 ml was then placed in the cavity slide and were incubated for 25±2ºC in a moist chamber in order to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were observed using stereoscopic microscope after 24 h. Furthermore, the percent germination of spores for each treatment was calculated using the formula (Eqn. 1 and Eqn. 2) [25].

$$\text{Percent spore germintion (\%)} = \frac{\text{No. of spores germinated}}{\text{Total no of spores examined}} \times 100$$

Eqn. 1

$$\text{Inhibition of spore germination (\%)} = \frac{\text{Gc}-\text{Gt}}{\text{Gc}} \times 100$$

Eqn. 2

Where Gc and Gt represent the mean number of germinated conidia in control and treated plates, respectively.

Agar well diffusion assay

The antifungal activity of the n-Hexane extract of *Rhizopogon species* was determined by agar well diffusion method [26]. About 7-8 days old fungal cultures grown on potato dextrose agar (PDA) medium were used to check the antifungal activity. An aliquot of

0.02 ml of inoculum from each fungal pathogen was inoculated in 20 ml of molten Saboured dextrose agar (SDA) medium in culture tubes. The culture tubes were then homogenized between the hands and poured into 90mm Petridishes. For solidification purpose culture plates were maintained under laminar airflow chamber and then wells were made using 5mm standard cork borer. Different concentrations of n-hexane extract viz. 100 mg/ml, 150 mg/ml and 200 mg/ml were prepared and added to respective wells. Nystatin 0.1 mg/ml (50 µl/disc) was used as standard (Positive control). The plates were then sealed and incubated at 25±2ºC for 2 to 3 days. The antimycotic activity of test n-Hexane extract was accessed by measuring the inhibition zone using standard scale [27].

RESULTS

GC-MS Analysis

The GC-MS results revaled (Fig. 3 and Fig. 4, Table. 1 and Table. 2) that n-hexane extract of *Rhizopogon* species shows the presence of 37 volatile and semi-volatile compounds. These identified compounds with their names and relative percentage by match factor and mass analysis are shown below.

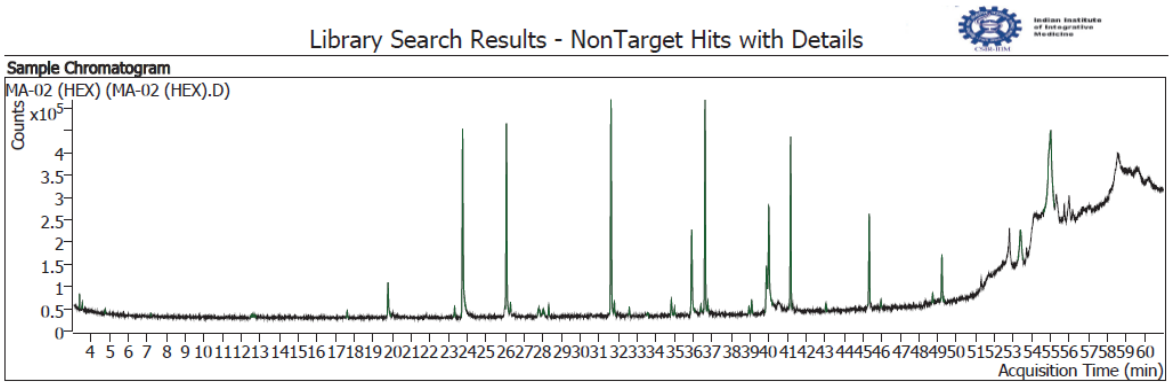

Fig. 3. *GC-MS chromatogram of n-Hexane extract of Rhizopogon species*

Table 1. *GC-MS profiling of 37 compounds from n-Hexane extract of Rhizopogon species with their names, retention time, chemical formula, percent area and match factor*

Component RT	Compound Name	CAS#	Formula	Component Area	Match Factor	Area %
3.3896	Pentane, 1-ethoxy-	17952-11-3	C7H16O	38635.4	78.0	0.1855
3.5380	Toluene	108-88-3	C7H8	25119.1	77.3	0.1206
4.7547	Benzene, chloro-	108-90-7	C6H5Cl	16508.8	74.4	0.07924
7.1763	1-Decanol	112-30-1	C10H22O	9212.0	79.0	0.04422
12.5477	Naphthalene	91-20-3	C10H8	32329.6	77.7	0.1552
12.6783	Acetic acid, trifluoro-, nonyl ester	30767-14-7	C11H19F3O2	34071.2	80.6	0.1635
17.6165	Cyclohexasiloxane, dodecamethyl-	540-97-6	C12H36O6Si6	49247.7	92.5	0.2364
19.7829	1-Tetradecene	1120-36-1	C14H28	291711.9	96.6	1.4
23.3263	Cycloheptasiloxane, tetradecamethyl-	107-50-6	C14H42O7Si7	69681.9	90.6	0.3345
23.7477	2,4-Di-tert-butylphenol	96-76-4	C14H22O	1659163.4	98.5	7.964
26.0743	Cetene	629-73-2	C16H32	1421860.7	97.8	6.825
26.2939	Hexadecane	544-76-3	C16H34	55633.2	87.3	0.267
27.7955	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]-	19435-97-3	C15H26O	105753.9	89.4	0.5076
28.0270	.alpha.-Cadinol	481-34-5	C15H26O	65392.0	81.8	0.3139
28.3238	Cyclooctasiloxane, hexadecamethyl-	556-68-3	C16H48O8Si8	67352.8	86.2	0.3233
31.6357	1-Octadecene	112-88-9	C18H36	1585593.4	98.1	7.611
31.8078	Octadecane	593-45-3	C18H38	98524.8	86.5	0.4729
32.6031	Cyclononasiloxane, octadecamethyl-	556-71-8	C18H54O9Si9	53367.5	77.9	0.2562
33.5765	Phthalic acid, 2-cyclohexylethyl butyl ester	1000309-08-4	C20H28O4	27988.8	79.4	0.1344
34.8348	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	1117-52-8	C18H30O	141187.0	88.6	0.6777
35.0129	Hexadecanoic acid, methyl ester	112-39-0	C17H34O2	52599.5	79.9	0.2525
35.9210	n-Hexadecanoic acid	57-10-3	C16H32O2	761272.5	93.4	3.654
36.4077	Cyclodecasiloxane, eicosamethyl-	18772-36-6	C20H60O10Si10	40412.9	71.9	0.194
36.6332	1-Nonadecene	18435-45-5	C19H38	1463600.0	97.5	7.025
36.7757	Eicosane	112-95-8	C20H42	76344.0	81.4	0.3665
38.9717	9,12-Octadecadienoic acid, methyl ester	2462-85-3	C19H34O2	50986.6	78.4	0.2447
39.1082	6-Octadecenoic acid, methyl ester, (Z)-	2777-58-4	C19H36O2	97575.5	86.6	0.4684
39.8976	9,12-Octadecadienoic acid (Z,Z)-	60-33-3	C18H32O2	450197.0	72.1	2.161
40.0163	cis-Vaccenic acid	506-17-2	C18H34O2	1126773.0	95.0	5.409
41.1797	Behenic alcohol	661-19-8	C22H46O	1108933.7	97.7	5.323
43.0552	Tetracosamethyl-cyclododecasiloxane	18919-94-3	C24H72O12Si12	51806.6	77.2	0.2487
45.3581	n-Tetracosanol-1	506-51-4	C24H50O	624962.5	95.3	3
45.9813	Tetracosamethyl-cyclododecasiloxane	18919-94-3	C24H72O12Si12	49052.8	72.1	0.2355
48.7234	Tetracosamethyl-cyclododecasiloxane	18919-94-3	C24H72O12Si12	70706.0	71.6	0.3394
49.2220	n-Tetracosanol-1	506-51-4	C24H50O	360912.8	87.4	1.732
53.3945	Stigmasterone	1000430-63-2	C29H46O	531681.4	76.5	2.552
55.0030	Urs-12-en-28-al, 3-(acetyloxy)-, (3.beta.)-	86996-88-5	C32H50O3	2347595.9	86.5	11.27

Table 2. *Major compounds identified from n-Hexane extract of Rhizopogon species*

Retention Time	Compound Name	Mol. Formula	Peak Area %
19.7829	1-tetradecene	$C_{14}H_{28}$	1.4
23.7477	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	7.964
26.0743	Cetene	$C_{16}H_{32}$	6.825
31.6357	1-octadecene	$C_{18}H_{36}$	7.611
35.9210	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	3.654
36.6332	1-nonadecene	$C_{19}H_{38}$	7.025
40.0163	cis-vaccenic acid	$C_{18}H_{34}O_2$	5.409
41.1797	Behenic alcohol	$C_{22}H_{46}O$	5.323
45.3581	n-tetracosanol	$C_{24}H_{50}O$	3

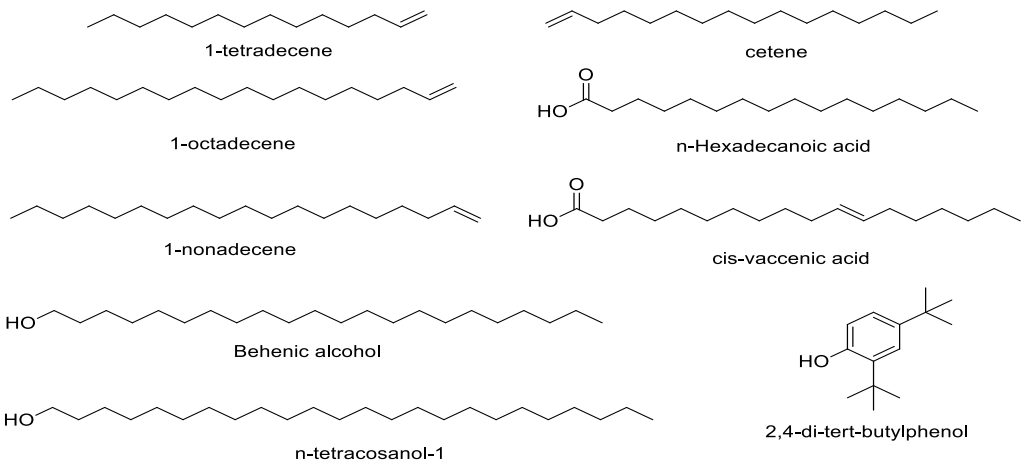

Fig. 4. *Structures and names of major compounds identified from n-Hexane extract of Rhizopogon species*

The compounds identified from n-hexane extract of *Rhizopogon species* were acids (8), polymers of silicon (7), alcohol (5), alkene (4), substituted benzene (3), alkane (3), polycyclic hydrocarbons (2), steroids (2), acyclic olifins (1), carbonyl compounds (1) and terpenoids (1).

Identification of fungi causing fungal rot of Apple

The present study revealed that apple rot in storage houses, markets and godowns is caused by *Penicillium chrysogenum* Thom (1910), *Aspergillus niger* Van Tieghem (1867) and *Alternaria alternata* (Fr.) Keissl. (1912).

Antifungal assay

Effect of different concentrations of n-Hexane extract of Rhizopogon species on the germination of spores of some fungal pathogens

It was revealed from the results (Fig. 5, Table. 3) that different concentrations of n-Hexane extract of *Rhizopogon* species brought about significant inhibition of spore germination of all the tested fungal pathogens. However, inhibition in spore germination increased with the increase in the concentration of n-Hexane extract. The maximum spore germination inhibition was found by highest concentration (200 mg/ml) against *Penicillium chrysogenum* (87.80 %) followed by *Aspergillus niger* (71.08 %) and *Alternaria alternata* (55.10 %) at same concentrations. Other concentrations also brought about spore germination inhibition but to a lesser extent. The inhibition in spore germination of *Penicillium chrysogenum* in different concentrations was 41.91 %, 58.08 % and 87.80 % respectively. Similarly, inhibition in spore germination of *Aspergillus niger* and *Alternaria alternata* in different concentrations were 36.00 %, 43.80 %, 71.08 % and 22.08 %, 33.33 %, 55.10 % respectively.

Table 3. *Effect of n-Hexane extract of Rhizopogon species on spore germination % & (Spore Inhibition %) against different fungi*

Fungal Pathogen	Spore germination %			
	100 mg/ml	150 mg/ml	200 mg/ml	Standard
Penicillium Chrysogenum	14.75 ± 0.57 (41.91)	12.14 ± 0.67 (58.08)	07.16 ± 0.58 (87.80)	46.26 ± 1.00
Aspergillus Niger	16.79 ± 0.52 (36.00)	13.90 ± 0.57 (43.80)	09.00 ± 1.54 (71.08)	76.90 ± 1.00
Alternaria Alternata	32.85 ± 1.00 (22.08)	28.44 ± 0.58 (33.33)	11.35 ± 2.00 (55.10)	87.49 ± 1.54

Values are represented as mean ± SD
Figures in parenthesis indicate the inhibition in spore germination (%)

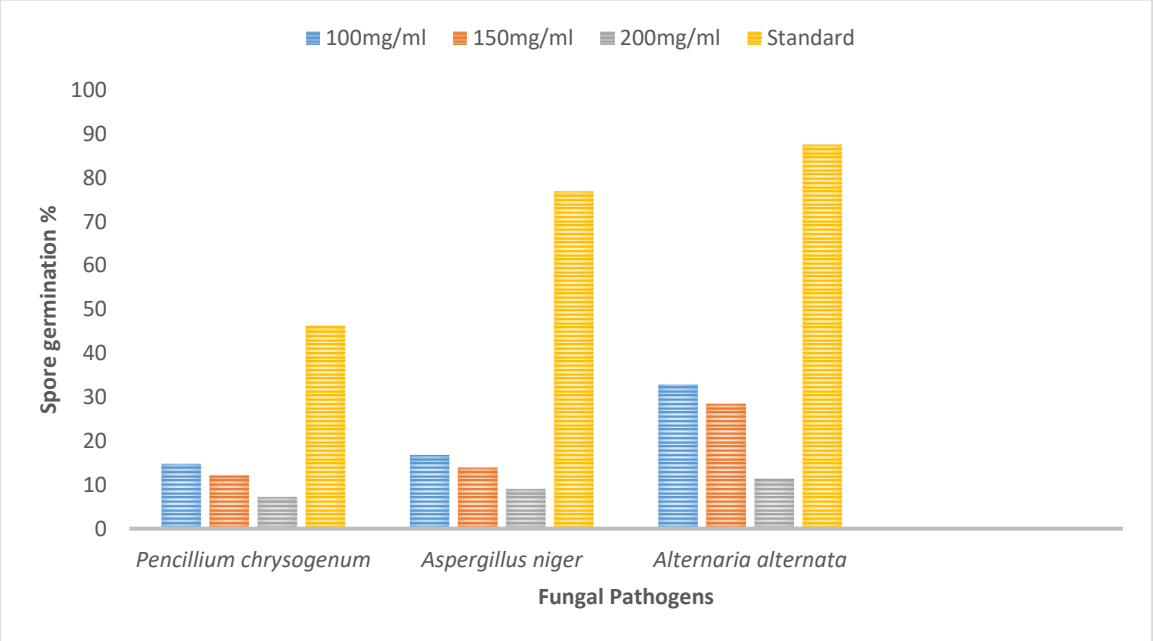

Fig. 5. *Effect of different concentrations of n-Hexane extract of Rhizopogon species on the spore germination of some fungal pathogens*

Agar well diffusion assay

Antifungal activity of n-Hexane extract of *Rhizopogon* species was accessed by measuring the reduction in zone of inhibition for different fungal pathogens. Significant zone of inhibition was observed in case of all the concentrations against the tested fungal pathogens. However, highest inhibition zone was observed in case of the highest concentration of n-Hexane extract (Fig. 6 and 7, Table. 4). The maximum inhibition zone was observed at highest concentrations against *Penicillium chrysogenum* (27.00 ± 1.00 mm), followed by *Aspergillus niger* (22.67 ± 2.08 mm) and *Alternaria alternata* (18.57 ± 1.52 mm). The reduction in zone of inhibition of *Penicillium chrysogenum* in different concentrations of extract was 21.00 ± 1, 24.33 ± 1.52 and 27.00 ± 1.00 respectively. Likewise, the reduction in zone of inhibition at different concentrations of n-Hexane

extract against *Aspergillus niger* was 17.00 ± 1.73, 19.33 ± 1.52, 22.67 ± 2.08 and in case of *Alternaria alternata* was 15.33 ± 1.52, 16.67 ± 1.52, 18.57 ± 1.52 respectively.

Table 4. *Zone of inhibition by n-Hexane extract of Rhizopogon species against different fungi*

Fungal Pathogen	Zone of Inhibition (mm) 100mg/ml	150 mg/ml	200 mg/ml	Standard
Penicillium Chrysogenum	21.00 ± 1.00	24.33 ± 1.52	27.00 ± 1.00	33.00 ± 1.00
Aspergillus niger	17.00 ± 1.73	19.33 ± 1.52	22.67 ± 2.08	33.67 ± 1.52
Alternaria alternata	15.33 ± 1.52	16.67 ± 1.52	18.57 ± 1.52	32.00 ± 1.00

Values are represented as mean ± SD

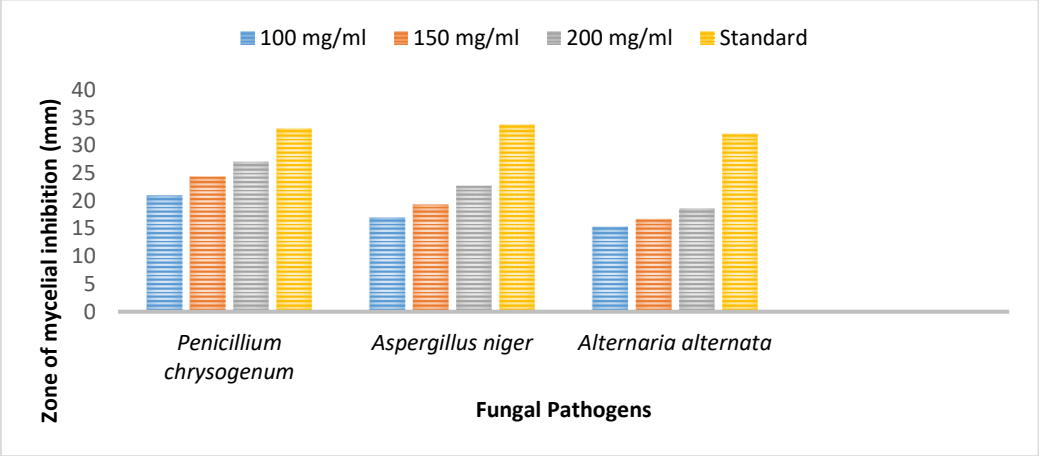

Fig. 6. *Effect of different concentrations of n-Hexane extract of Rhizopogon species on the zone of mycelial inhibition of some fungal pathogens*

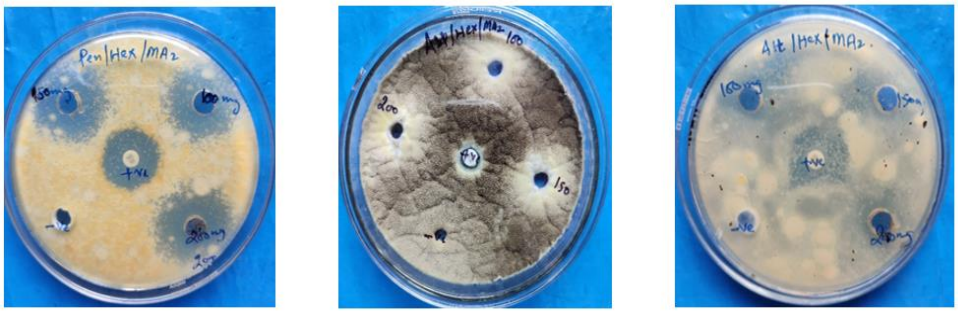

Fig. 7. *Zone of inhibition by n-Hexane extract of Rhizopogon species against (A) Penicillium chrysogenum, (B) Aspergillus niger, (C) Alternaria alternata*

DISCUSSION

It is revealed from the GC-MS results that 37 volatile and semi-volatile compounds have been reported for the first time and identified from the n-Hexane extract of *Rhizopogon* species which consists of acids (8), polymers of silicon (7), alcohol (5), alkane (3), alkene (3), polycyclic hydrocarbons (2), steroids (2), acyclic olifins (1), carbonyl compounds (1) and terpenoids (1). Volatile profiles of eight macrofungi were characterized by gas chromatography-mass spectrometry in which 11 alcohols, 11 ketones, 15 aldehydes, 3 sulfur compounds and alkenes, 8 terpenes, 7 acid and esters, 5 heterocyclic compounds, 20 aromatic compounds, and 4 other compounds were identified [28]. The present study was also supported by the work of [29, 30, 31, 32].

Natural compounds with biological activity are normally present in plants, mushrooms and other natural sources. Macrofungi need antimycotic and antibacterial compounds to survive in their natural habitats. Therefore, antifungal agents with more or less potent antimycotic properties could be isolated from various macrofungi that may be beneficial for mankind [30]. It was revealed from the present study that n-hexane extract of *Rhizopogon* species shows the presence of various major bioactive compounds viz. 1-tetradecene, 2, 4-di-tert-butylphenol, Cetene, 1-octadecene, n-Hexadecanoic acid, 1-nonadecene, cis-vaccenic acid, Behenic alcohol and n-tetracosanol which are thought to be responsible for its strong antifungal activity against all the test fungal pathogens such as *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata.* Similarly, antifungal activity of various mushroom extracts against different fungal pathogens like *Aspergillus fumigatus, Aspergillus niger, Alternaria alternata, Botrytis cinerea, Aspergillus versicolor, Penicillium funiculosum and Penicillium ochrochloron* was also reported by [32, 33, 34, 35]. Reis et al. [36] reported that *Agaricus bohusii* shows antifungal activity against *Penicillium verrucosum* which also support our work.

From very beginning, we consistently observed a variety of decay-causing fungi like *Penicillium* spp. (blue mold), *Botrytis* spp. (gray mold), *Alternaria* spp. (*Alternaria* rot), *Mucor* spp. (Mucor rot), *Colletotrichum* spp. (bitter rot), and *Botryosphaeria* spp. (white and black rots) causing postharvest fungal rot of various rosaceous fruits. The present study is in conformity with the work of [18, 19, 20] who reported that apple rot is caused by various pathogenic fungi such as *Penicillium* species, *Alternaria alternata*, *Fusarium* species, *Botrytis cinerea*, *Mucor* species, *Rhizopus stolonifer* etc. that are common and causing significant economic losses to farmers and apple industry across the world.

CONCLUSION

It is concluded from the present study that both volatile and semi-volatile compounds were identified for the first time from the n-Hexane extract of the novel species of truffle like mushroom *Rhizopogon* species growing in Kashmir Valley. The isolated and identified copmounds belonged to different types of compounds like acids, polymers of silicon, alcohol, alkane, alkene, polycyclic hydrocarbons, steroids, acyclic olifins, carbonyl compounds and terpenoids. The n-Hexane extract of novel *Rhizopogon* species shows strong antimycotic activities against all rot causing fungi on apple. Hence, it may be used as strong antifungal agent as biofungicide in near future after further investigation. The present study will help in developing the proper management strategies for decreasing disease incidance and controlling the postharvest fungal rot of apple as well as for proper preservation of the apple.

Acknowledgement. The authors are grateful to the Head, Department of Botany, University of Kashmir and IIIM, Jammu for providing necessary help and facilities during the course of the study. The first author is also thankful to CSIR, New Delhi for providing financial assistance.

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