Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 02 (Mar–Apr 2024); pp 390–394

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Prevalence of Bioactive Compounds in *Mycothermus thermophilus* Indigenous Culture under Thermophilic Condition

Rekha Gupta*[1] and Pragya Kulkarni[2]

[1-2] Department of Microbiology, Government V. Y. T. P/G Autonomous College, Durg - 491 001, Chhattisgarh, India

Received: 04 Jan 2024; Revised accepted: 02 Mar 2024; Published online: 18 Mar 2024

Abstract

The Present study aimed to isolate, characterize and identify thermophilic fungi from dairy farm compost soil and screen for potential of secondary metabolites production. Dairy farm compost soil from the Bhilai Durg region of Chhattisgarh, was used for primary isolation of fungi and further identified as *Mycothermus thermophilus* using internal transcribed spacer (ITS) technique. The Phylogenetic tree was inferred using Neighbour- joining method. The evolutionary distances were computed using the maximum composite likelihood method. The secondary metabolites found in the CFCF were analyzed by GCMS and identified the presence of some important chemical compounds such as lactic acids, oximes, maltol, pyrrolo(pyrazine1,4 dione), 2,5 piperzinedione etc. Results suggest that dairy farm compost soil has thermophilic fungal species that could be a potential source of these useful chemical compounds which has diverse industrial significance.

***Key words*:** Thermophilic, Metabolite, Bioactive compound, Phylogenetic, Pyrrolo, Piperzinedione, Oximes, Maltol

Thermophilic fungi are magnificent source for novel thermostable enzymes which are essential for biotechnological and industrial applications [1]. These fungi can grow in extremely harsh temperature ranges including 20-50°C. Thermophilic fungi can survive in extreme heated regions, such as deep sea, hydrothermal vents, hot springs, and volcanic environments, and enabling their probability to produce novel and bioactive secondary metabolites. Many of them have a well-developed secondary metabolism system resulting alternative pathways for degradation of available complex organic matter. These isolates are sources of industrially important enzymes like pectinases, laccases, xylanases, proteases and cellulases etc. Thermophilic fungi constitute a diverse group of organisms and their prevalence has also been reported from various substrates such as fungal compost, municipal waste, manure and coal mine soil, they have remained unexplored compared to *Thermophilic eubacteria* and archaea [2-3]. One of the remarkable features of thermophilic fungi is their well-developed secondary metabolism system, which often leads to the synthesis of diverse bioactive compounds. These compounds may have applications in pharmaceuticals, agriculture, and various other industries [4]. Additionally, thermophilic fungi possess alternative pathways for the degradation of complex organic matter, making them valuable players in nutrient cycling and organic material decomposition. Among their diverse metabolic capabilities, thermophilic fungi are notable producers of industrially important enzymes such as pectinases, laccases, xylanases, proteases, and cellulases. These enzymes exhibit remarkable stability and activity at high temperatures, making them ideal candidates for various industrial processes, including food processing, biofuel production, waste treatment, and bioremediation [5-7].

Mycothermus thermophilus is one of the ubiquitous thermophilic fungi in compost and soil. Although in survey of thermophilic fungi, one of the most common species found is *Scytalidium thermophilum* (Chaetomiaceae). Its significant ecological and economic implications have also been reported [8]. Nearly all living forms, including eukaryotes (plants, animals), prokaryotes (bacteria, cyanobacteria), and fungi, are known to produce bioactive compounds [9]. Bioactive compounds represent a fascinating and diverse group of molecules with the potential to enhance human health and wellbeing. They are abundantly found in plants but fungi and some other organisms are also known to produce a wide array of bioactive compounds with diverse chemical structures and biological activities. These compounds play important role in the physiology and ecology of fungi representing antibiotic, antifungal, immunosuppressant, mycotoxic and antioxidant properties [10]. Fungi, in particular, are prolific producers of bioactive compounds, which play crucial roles in their physiology and ecology. These compounds serve various functions such as defense against competitors, predators, or pathogens, communication between organisms, and aiding in nutrient acquisition.

Nowadays, researchers are emphasizing on begun on the analysis of the bioactive components of thermophilic fungus. Their research revealed a variety of secondary metabolites, including polyketides, alkaloids, and peptides with remarkable cytotoxic and antibacterial properties. The increasing need for bioactive compounds with antibacterial, antifungal and anticancer effects requires further research into rare

***Correspondence to:** Rekha Gupta, E-mail: rekha7987@gmail.com; Tel: +91 8982608438

Citation: Gupta R, Kulkarni P. 2024. Prevalence of bioactive compounds in *Mycothermus thermophilus* indigenous culture under thermophilic condition. *Res. Jr. Agril. Sci.* 15(2): 390-394.

microorganisms [11]. In this paper dairy composting site was explored for high temperature tolerating *Mycothermus thermophilus* fungal species and studied as possible source for bioactive compounds.

MATERIALS AND METHODS

The investigation included collection of dairy farm compost soil samples from Gokul Nagar Bhilai, Chhattisgarh for isolation of thermophilic fungi, morphological and molecular characterization and GC-MS analysis of fungal extract for presence of bioactive compounds.

Collection of soil samples

Dairy farm compost soil sample were randomly collected from four different sites of Gokul Nagar, Bhilai region of Chhattisgarh, India. Soil sample was taken from 5-10cm deep layer of soil in sterilized polythene bags and brought into laboratory for isolation of fungi.

Fungus isolation

The collected soil samples were pretreated at 45 °C to activate the thermophilic fungi and to reduce the chances of mesophilic ones. Soil sample were inoculated using serial dilution method. The petri plates were incubated at 50 °C for a period of 4-6 days. During incubation period fungal growth were regularly observed. Isolated fungal culture was mounted with lactophenol cotton blue stain and observed under microscope for morphological characters.

Morphological characterization

The isolates were observed for macroscopic as well as microscopic characters like colony colour, reverse coloration, hyphal structure, septation, shape and spore bearing structure, pigmentation were observed [12-13].

Molecular characterization

Genomic DNA was isolated from the sample. The ITS1 –5.8S—ITS2 rDNA was amplified using primers ITS4 (forward primer) and ITS5 (reverse primer) [14]. Amplification was achieved in a vial containing 10x buffer, $MgCl_2$ 15 mM, dNTP 0.2 mmol, Forward primer and Reverse primer 10 picomolar, Taq Polymerase 2µl, DNA sample 50-100 ng/µl and Milli Q water. The PCR reaction was carried out using a Thermal Cycler with conditions as follows: Initiation for 10 minutes at 94 °C, denaturation for one minute at 94 °C, annealing for 30 seconds at 55 °C, extension for 1 minute at 72 °C. The Thermal Cycler was run for 35 cycles and then a final extension cycle was run at 72 °C for 10 minutes. The PCR products were analyzed by electrophoresis on 1.2% agarose. The fungal strain was identified according to a molecular biological protocol by DNA amplification and sequencing of the internal transcribed spacer (ITS) region. After alignment into GenBank database, the similarities of the target sequences were analyzed to identify the fungi and its closest neighbors.

Liquid culture fermentation and crude extract preparation

The fungal strain was cultivated on YPSs broth medium. The flasks were incubated at 50 °C for 6 days with a stirring speed of 150 rpm. The cultures were centrifuged at 3000 rpm for 30min. to obtain cell free culture filtrate. The ethyl acetate extract of thermophilic fungi was prepared according to the modified method of Raviraja and Shridhar [15]. Ethyl acetate extraction was carried out by adding ethyl acetate in ratio of 1:1 with the volume of supernatant. Then the culture filtrate was taken in separating funnel and shaken vigorously for 15 min. The solutions were then allowed to stand, the organic phase of solvents so obtained, were collected. The solvent ethyl acetate was evaporated and the resultant residue was dried in vacuum evaporator to yield the crude extract (Culture filtrate extract) which further subjected to GC-MS analysis.

GCMS analysis

Sample was dissolved in Ethyl Acetate and injected in an GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Splitless. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature 45°C for 2 min then 140°C at 5°C/ min and finally increased to 280°C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out 70 eV. The running time of the GC was from 9.10 min – 52.0 min. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L).

RESULTS AND DISCUSSION

Morphological characterization

Four different sites: site A, site B, site C, site D were selected in Gokul Nagar Bhilai for isolation of *Mycothermus thermophilus*. Maximum number of colonies was observed in site D followed by site B, site A and site C respectively (Table 1) fungal Colony was greyish black in colour. Hyphae were septate and colourless and conidia were smooth walled and globose shaped (Fig 1-2).

Table 1 Occurrence of *Mycothermus thermophilus* in Gokul Nagar, Bhilai site

S. No.	Name of site	No. of colonies observed
1.	Site A	05
2.	Site B	07
3.	Site C	03
4.	Site D	09

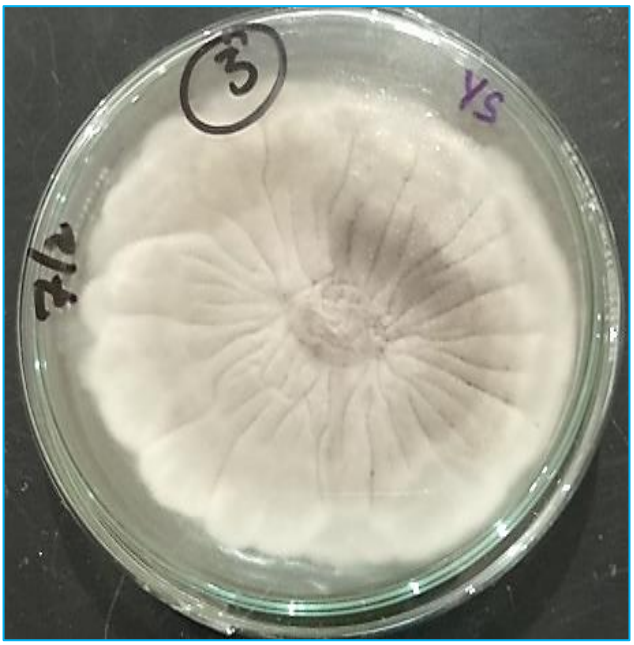

Fig 1 Culture plate showing colony of *Mycothermus thermophilus*

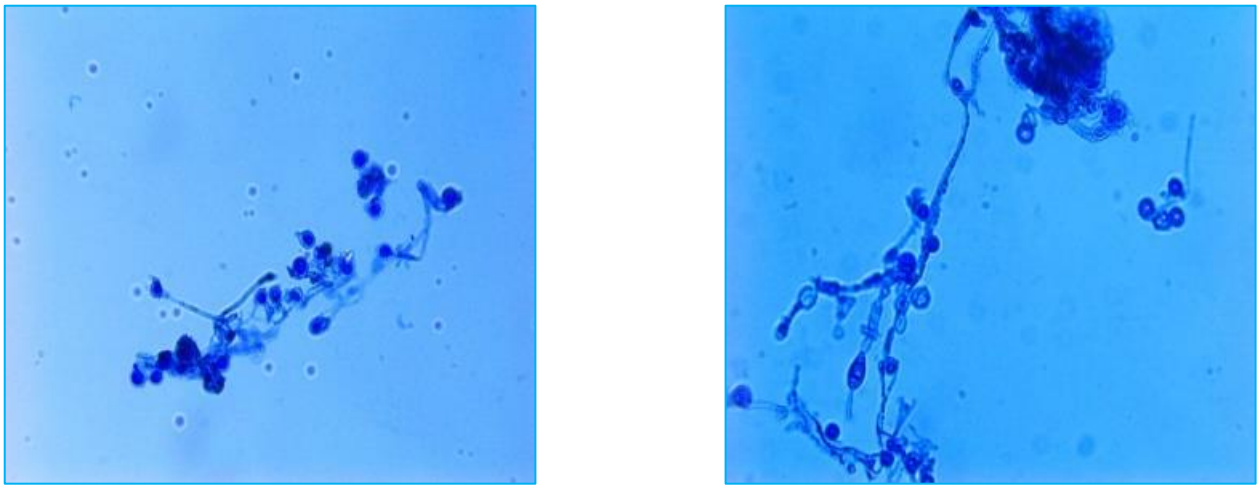

Fig 2 Fungal isolate showing septate hyphea, branching conidiophores and conidia

Molecular characterization

Aligned Sequence of isolated Fungi (628 bp)

TGGAAGTATAAGTGATAACAATGTGTCCATTGGTGAATCAGCGGAGGGATCAGGTAACAGGAGTTACACAACTC
ACCAAACCATTGTGAACCGACCCTTTCTGTTGCTTCGGCGGGAGGCGCCTCGGCGCCCCTCGGCCGATCCCCCGCC
CCCGCGGCGTGGGGGGAGGCTCCCGCCGGAGGAATACCCCAAACCCTTTGCAGCACATGGCCTCTCTGAGTTTAT
GTACTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCGGTATTCCGGCGG
GCATGCCTGTTCGAGCGTCATTTCAACCATCAAGCCCTGGGCTTGTGTTGGGGACCTGCGGCTGCCGCAGGCCCTG
AAATGCAGTGGCGGGCTCGCAAGACACACCGAGCGTAGTAGCTTACAACCTCGCTTTGGGAGTGCTGCGGGTGCT
GGCCGTAAAGCGACCTTTGCGTCTTTAGCACTAGGTTGACCTCGGATCACGGTAGGGAGGACGCTCGGCGTGAAC
TTAAGCATATCAAAAACCGGGAGGAAA

The culture was identified as *Mycothermus thermophilus* showing highest similarity (96.64%) with *Mycothermus thermophilus* isolate DB13 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer with accession number MN386257.1

The bioactive compounds of ethyl acetate extracts of thermophilic fungi were identified by GC-MS analysis and the results were presented in the (Table 2). The GC-MS chromatogram of ethyl acetate extract of *Mycothermus thermophilus* revealed the presence of various compounds with corresponding peaks at different retention times in (Fig 4). Literature comparison of mass spectra was used in compound identification [16-19]. A total of 27 different compounds were identified in isolated fungal isolate extract. Pyrrolo (pyrazine1,4dione) (19.41%) was the most abundant compound followed by 2,4 Piperazine dione (11.83%), Harmaline (10.51%) and Fumigaclavin C (10.44%) while Tridecanoic acid (0.29%) was the least in proportion. The extract also comprised of other groups of compounds such as hexestrol, oxime, benzoic acid, ascorbic acid, hexadecenoic acid, octadecanoic acid etc.

Fig 3 BLAST analysis showing phylogenetic tree of *Mycothermus thermophilus* along with closest relatives

GCMS analysis

Table 2 Metabolite profile for thermophilic fungal isolate

S. No.	Name of compound	Peak area %	Retention time
1	Oxime-, methoxy-phenyl-_	2.97	7.748
2	2,5-Dihydroxybenzaldehyde, 2TMS derivative	0.52	13.144
3	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	1.20	16.629
4	Tridecanoic acid, methyl ester	0.29	29.219
5	3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo	4.73	31.046
6	Pyrrolo[1,2-a]pyrazine-1,4-dione	19.41	31.832
7	Benzoic acid, 3-(2-methoxyethyl)heptyl ester	0.44	32.391
8	Cyclo(L-prolyl-L-valine)	0.76	32.635
9	3-Heptyne-2,5-diol,	1.00	33.203
10	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol	1.05	33.520

11	2,2-Dimethyl-1-(3-oxo-but-1-enyl)-cyclop	0.59	33.957
12	Hexadecanoic acid, methyl ester	0.72	34.031
13	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	0.67	34.170
14	Octahydrodipyrrolo[1,2-a:1',2'-d]pyrazine-5,1	0.53	34.292
15	l-(+)-Ascorbic acid 2,6-dihexadecanoate	1.29	34.505
16	5,5-Dimethyl-3-(4-morpholinylmethyl)	7.56	34.685
17	2,5-Piperazinedione	11.83	37.934
18	Pyrazino[1,2-a]indole-1,4-dione	1.41	39.061
19	1,3,6-Triazahomoadamantane	0.61	40.944
20	4-Hydroxy-7-(trifluoromethyl)quinoline,	3.28	41.425
21	9-Octadecenoic acid, methyl ester,	0.65	42.682
22	1H-Azepino[5,4,3-cd]indole-4-carboxylic acid	0.54	43.123
23	Harmaline	10.51	43.471
24	Hexestrol, O,O'-di(trifluoroacetyl)-	4.62	44.133
25	Fumigaclavine C	10.44	45.050
26	Tetracosamethyl-cyclododecasiloxane	3.01	45.266
27	Squalene	3.75	45.696

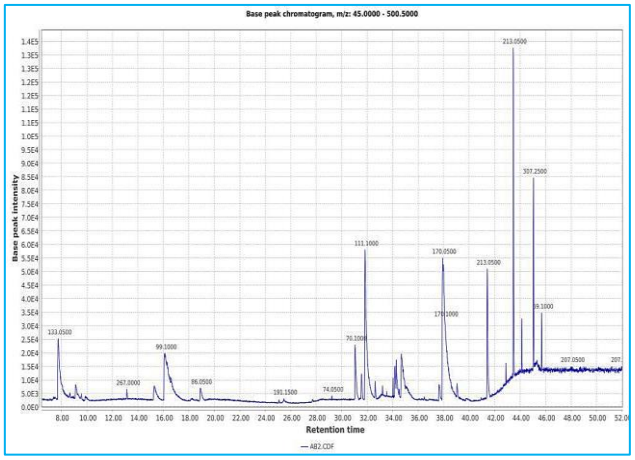

Fig 4 GCMS chromatogram of *Mycothermus thermophilus*

Recently a few researchers begun to focus on the investigation of thermophilic fungus bioactive components. Their findings demonstrated a wide range of secondary metabolites with exceptional cytotoxic and antibacterial properties, such as polyketides, alkaloids, and peptides. More investigation into rare microbes from unusual and unexplored environments is necessary to meet the growing need for bioactive chemicals with antibacterial, antifungal, and anticancer properties. Alrumman *et al*. [20] showed that 50 fungi isolates have 40 bioactive compounds capable of fighting pathogenic microbes. There are more than 20,000 microbial derived bioactive metabolites with a range of bioactive characteristics [21]. The nature of identified bioactive compounds of *Mycothermus thermophilus* are phenolic compounds, fatty acids, sugar alcohols, terpenoids, aldehydes, ketides, steroids and aroma compounds.

Based on the ethno-botanical database, Gopalkrishnan and Udaykumar [22] suggested, that compounds such as furan, phenols, flavonoids and fatty acid esters possess antioxidant, antibacterial, antimicrobial, anti-inflammatory, anti-proliferative, anticancer, antitumor, antidiabetic, antiarthritic and antimalarial activities. The compounds furanose, phenols, flavonoids, alkaloids, steroids, and fatty acid esters were also found in the ethyl acetate extracts of isolated fungi in the present investigation. Many fungal secondary metabolites with diverse chemical structures and a wide range of biological activities were discussed by Suryanarayanan *et al.* [23]. In this study, we identified fungus capable of producing antimicrobial bioactive compounds and identify the bioactive components using gas chromatography-mass spectrometry (GC-MS) analysis.

CONCLUSION

Research into alternative sources of bioactive compounds and metabolic capabilities of *Mycothermus thermophilus* indicated its significant potential for pyrrolo, piperazinedione, harmaline and fumigaclavin under thermophilic conditions. A total of 27 bioactive compounds were detected in *Mycothermus thermophilus*. Further investigations into the quantification of the metabolites in this study are still needed, particularly for those significant metabolites obtained in this fungal extract.

Acknowledgement

The authors are thankful to department of Microbiology, Govt. V.Y.T. P/G Autonomous College, Durg for providing necessary research facilities and also thankful to Cytogene research and development centre, Lucknow for molecular identification.

LITERATURE CITED

1. Moretti M, Bocchini-Martins DA, Silva RD, Rodrigues A, Sette LD, Gomes E. 2012. Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. *Brazilian Journal of Microbiology* 43: 1062-1071.
2. Al-Dhabi NA, Esmail GA, Duraipandiyan V, Valan Arasu M, Salem-Bekhit MM. 2016. Isolation, identification and screening of antimicrobial thermophilic Streptomyces sp. Al-Dhabi-1 isolated from Tharban hot spring, Saudi Arabia. *Extremophiles* 20: 79-90.
3. Elkhateeb WA, Kolaibe AG, Elnahas MO, Daba GM. 2021. Highlights on Chaetomium morphology, secondary metabolites and biological activates. *Journal of Pharmaceutics and Pharmacology Research* 4(1): 1-5.
4. Yang XY, Zhang JX, Ding QY, He ZC, Zhu CY, Zhang KQ, Niu XM. 2020. Metabolites from two dominant thermophilic fungal species *Thermomyces lanuginosus* and *Scytalidium thermophilum*. *Chemistry and Biodiversity* 17(5): e2000137.

5. Orfali R, Perveen S. 2019. New bioactive metabolites from the thermophilic fungus *Penicillium sp*. isolated from Ghamiqa hot spring in Saudi Arabia. *Journal of Chemistry* 2019: 7162948. 1-7.
6. Cooney DG, Emerson R. 1964. An account of their biology, activities, and classification. *In*: Thermophilic Fungi. Ed. W. H. Freeman and Company, San Francisco. pp 27.
7. Maheshwari R, Bharadwaj G, Bhat MK. 2000. Thermophilic fungi: their physiology and enzymes. *Microbiology and Molecular Biology Reviews* 64(3): 461-488.
8. Natvig DO, Taylor JW, Tsang A, Hutchinson MI, Powell AJ. 2015. *Mycothermus thermophilus* gen. et comb. nov., a new home for the itinerant thermophile *Scytalidium thermophilum* (Torula thermophila). *Mycologia* 107(2): 319-327.
9. Ibrar M, Ullah MW, Manan S, Farooq U, Rafiq M, Hasan F. 2020. Fungi from the extremes of life: An untapped treasure for bioactive compounds. *Applied Microbiology and Biotechnology* 104: 2777-2801.
10. Prasad GS. 2019. Pharmaceutical Applications of Thermophilic Fungi. *In*: *Fungi in Extreme Environments: Ecological Role and Biotechnological Significance*. pp 269-289.
11. Salano OA, Makonde HM, Kasili RW, Boga HI. 2018. Isolation and characterization of fungi from a hot-spring on the shores of Lake Bogoria, Kenya. *Journal of Yeast and Fungal Research* 9(1): 1-13.
12. Gilman JC. 1957. *A Manual of Soil Fungi*. 2nd Edition. Iowa State University Press, Inc.
13. Ainsworth GC, Bisby GR. 1995. *Dictionary of the Fungi*. 8th Edition. Common Wealth Mycological Institute, Kew, Surrey. pp 445.
14. White TJ, Bruns T, Lee SJ, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. California, USA: Academic Press. *San Diego* 18(1): 315-322.
15. Raviraja NS, Maria GL, Sridhar KR. 2006. Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. *English Life Science*. pp 6515-6520.
16. Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism—from biochemistry to genomics. *Nature Reviews Microbiology* 3(12): 937-947.
17. Conrado R, Gomes TC, Roque GSC, De Souza AO. 2022. Overview of bioactive fungal secondary metabolites: Cytotoxic and antimicrobial compounds. *Antibiotics* 11(11): 1604.
18. Kanjana M, Kanimozhi G, Udayakumar R, Panneerselvam A. 2019. GC-MS analysis of bioactive compounds of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*. *Journal of Biomed. Pharm. Science* 2: 123.
19. Ellis DH, Keane PJ. 1981. Thermophilic fungi isolated from some Australian soils. *Australian Journal of Botany* 29(6): 689-704.
20. Alrumman SA, Mostafa YS, Al-Qahtani ST, Sahlabji T, Taha TH. 2019. Antimicrobial activity and GC-MS analysis of bioactive constituents of Thermophilic bacteria isolated from Saudi hot springs. *Arabian Journal for Science and Engineering* 44: 75-85.
21. Berdy J. 2005. Bioactive microbial metabolites. *The Journal of Antibiotics* 58(1): 1-26.
22. Gopalakrishnan K, Udayakumar R. 2014. GC-MS analysis of phytocompounds of leaf and stem of *Marsilea quadrifolia* (L.). *International Journal of Biochemistry Research and Review* 4(6): 517-526.
23. Suryanarayanan TS, Hawksworth DL. 2005. Fungi from little-explored and extreme habitats. *Biodiversity of Fungi: Their Role in Human Life* 34: 33-48.