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 437–441

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

Biosynthesis of Silver Nanoparticle from Endophytic Fungi Characterization in FT-IR and GC-MS

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Received: 11 Sep 2023; Revised accepted: 16 Mar 2024; Published online: 30 Mar 2024

Abstract

In the current investigation, the isolation of endophytic fungi from medicinal plants associated with marine environments was proposed. Specifically, the plants *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Ceriops decandra, Excoecaria agallocha*, and *Rhizophora mucronata* were analyzed. A total of 16 endophytic fungi were isolated and identified. The fungi *Aspergillus flavus, A. fumand P. citrinumigatus, A. niger, A. terreus, A. ochraceous, Chaetomiun sp., Fusarium sp., F. moniliforme, F. oxysporum, Pencillium chrysogenum, P. citrinum, P. janthinellum, P. purpurrescens, R. stolonifer*, and *Trichoderma harzianum* were subjected to screening for protease enzyme activity using the in vitro method of plate assay. According to the screening results, *Trichoderma harzianum* exhibited a significant zone of clearance, indicating its effectiveness in inhibiting the growth of other organisms. The presence of authenticated functional groups of compounds, known for their diverse biological activities, was estimated. The compounds were analyzed using FT-IR, which allowed for the identification of amines, nitrogen, alkene, and halogen compounds. Additionally, GC-MS analysis was employed to identify volatile compounds and determine their functional properties. A total of ten bioactive compounds were analyzed using the GC-MS method, and these compounds were found to be responsible for various biological activities. Therefore, *Trichoderma harzianum* shows potential as fungi for the production of protease enzymes and for use in industrial processes in the future.

***Key words*:** Endophytic fungi, Protease enzyme, FT-IR, GC-MS, *Trichoderma harzianum*

Endophytes, predominantly comprising fungi and bacteria, establish colonization within the vital internal tissues of plants [1]. The Earth harbors an extensive array of approximately 300,000 distinct plant species, consequently giving rise to a diverse assemblage of cultivable and non-cultivable endophytic microorganisms within their communities [2-3]. Endophytic fungi are a group of microorganisms that inhabit various plant tissues, including leaves, stems, flowers, seeds, and fruits, without causing any apparent harm to the host plant [4]. Furthermore, endophytic fungi have been found to synthesize numerous secondary metabolites that possess significant advantages in the fields of medicine, pharmaceuticals, agriculture, and environmental applications [5]. The *P. oxalicum* LA-1 strain of endophytic fungi has the ability to generate *C. quinquefasciatus* [6].

Nanotechnology has emerged as a significant field of contemporary research, with notable advancements in the domains of electronics and medicine. Notably, the synthesis and utilization of nanoparticles below 100 nm in size have garnered considerable attention. This progress has given rise to a novel branch of nanotechnology, referred to as "green nanotechnology" or "nanobiotechnology," which integrates biological principles with physical and chemical methodologies to fabricate environmentally sustainable nano-sized particles possessing specific functionalities.

A diverse range of biological entities, such as plants, algae, fungi, yeast, bacteria, and viruses, can be employed for the biosynthesis of nanoparticles. In recent times, particular emphasis has been placed on investigating fungi as efficient biofactories for the production of silver nanoparticles. This preference stems from the multitude of bioactive properties exhibited by fungi, which hold immense potential for various biomedical applications [7].

This study reports on the biosynthesis of silver nanoparticles by an endophytic fungus that was isolated from the medicinal herb *Abrus precatorius*. The endophytic fungus was identified as *Phyllosticta owaniana* KUMBMDBT-32 (NCBI accession number MW007919). The fungus was cultivated using submerged fermentation and extracted with ethyl acetate (1:1 V/V). The extract was subjected to GC-MS analysis, which revealed the presence of 42 bioactive compounds as secondary metabolites. The synthesised silver nanoparticles were characterised using Fourier-transform infrared spectroscopy (FTIR). The absorption spectra obtained from bio spectrophotometric analysis showed a peak at 400 nm, which confirmed the synthesis of silver nanoparticles. An FTIR study showed peaks corresponding to different possible functional groups, confirming the reduction and formation of silver nanoparticles [8]. In contemporary times, gas chromatography-mass spectrometry (GC-MS) has emerged as

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Citation: Oviya P, Madhanraj P. 2024. Biosynthesis of silver nanoparticle from endophytic fungi characterization in FT-IR and GC-MS. *Res. Jr. Agril. Sci.* 15(2): 437-441.

a prominent technological platform for the analysis of secondary metabolite fingerprints in various species, including plants [9]. Diabetes represents a significant global health and economic challenge, distinguished by elevated blood sugar levels resulting from deficiencies in insulin production, insulin activity, or both. This condition impacts approximately 6 percent of the global population [10-11]. The chemical constituents of the ethyl acetate extract derived from *Coenophialum* mushrooms were subjected to analysis using the Gas Chromatography-Mass Spectrometry (GC-MS) technique.

MATERIALS AND METHODS

Collection of leaf sample

The present study involved the collection of various medicinal plant species, namely *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Ceriops decandra, Excoecaria agallocha* and *Rhizophora mucronata*. The collected plant samples were carefully placed in sterilized polythene bags, which were then securely sealed to ensure the preservation of the plant material during transportation to the laboratory.

Isolation and identification of plant sample

Endophytes were isolated from plant leaf samples [12] using a specific methodology. The surface sterilization technique was employed [13]. A Petri dish containing a mixture of potato dextrose agar (PDA) medium and amoxicillin (15mL/l) was prepared and incubated at a temperature of 25°C in complete darkness. After a period of 7 days, the emerging hyphae were transferred to fresh PDA medium for the purpose of purification and subsequent identification. The identification of fungal taxa was carried out according to established protocols outlined in authoritative references such as the Manual of Penicillia [14], Manual of soil fungi [15], Manual of Aspergilli [16], Hyphomycetes [17], and Dematiaceous Hyphomycetes [18].

Preparation and extraction of culture from Trichoderma harzianum [19]

Fungal biomass was generated through the aerobic cultivation of fungi in potato dextrose broth (PDB) and subjected to incubation at a temperature of 24 ± 1 ºC for duration of 1-7 days. Following the incubation period, the sponge mat was rinsed twice with double-distilled water, subsequently transferred to a flask containing 100 ml of sterile distilled water, and placed on an orbital shaker operating at a speed of 140 rpm for duration of 48 hours at a temperature of 24 ± 1 °C. After the 48-hour period, the resulting culture filtrate was filtered using Whatman No. 1 filter paper.

Silver nanoparticles using culture of T. harzianum [19]

The aqueous filtrate of *Trichoderma harzianum* was subjected to varying concentrations of aqueous silver nitrate solution, namely AgNO3 at concentrations of 0.5, 1.0, 1.5, and 2.0 µg/ml. Subsequently, the resulting mixture was incubated at room temperature for a duration of 1 to 3 days.

Characterizations of $AgNO_3$ [20]

The confirmation of $AgNO_3$ synthesis was achieved through the utilization of UV-160 V-visible spectroscopy. Subsequently, the composition of the nanoparticles was thoroughly investigated through the application of FTIR and GCMS techniques.

FT-IR spectroscopic analysis [21]

FTIR analysis was performed using Spectrophotometer system which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

GC-MS analysis [21]

The Shimadzu 2010 plus instrument, equipped with an AOC-20i auto sampler and gas chromatography interfaced to a mass spectrometer, was utilized for GC MS analysis under the following conditions: a RTX 5Ms column (0.32mm diameter, 30m length, and 0.50µm thickness) was employed in electron impact mode at 70eV. Helium gas (99.999%) was utilized as the carrier gas at a constant flow rate of 1.73 ml/min, and an injection volume of 0.5 µI was employed with a split ratio of 10:1. The injector temperature was set at 270 °C, while the ion-source temperature was set at 200 °C. The oven temperature was programmed to increase from 40 °C (isothermal for 2 min) at a rate of 8 °C/min to 150 °C, followed by an increase of 8 °C/min to 250 °C, and ending with a 20 min isothermal at 280 °C. Mass spectra were taken at 70eV with a scan interval of 0.5 seconds and fragments ranging from 40 to 450 Da. The total GC running time was 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The Turbo Mass Ver 5.2.0 software was utilized to handle mass spectra and chromatograms.

RESULTS AND DISCUSSION

The morphological identification of endophytic fungi resulted in the discovery of 15 distinct endophytic fungi originating from various parts of different plant species. These fungi were identified as *Aspergillus flavus*, *A. fumand*, *Pleurotus citrinumigatus*, *Aspergillus niger*, *Aspergillus terreus, Aspergillus ochraceous*, *Chaetomiun sp.*, *Fusarium sp.*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Pencillium chrysogenum*, *Pencillium citrinum*, *Pencillium janthinellum*, *Pencillium purpurrescens*, *Rhizopus stolonifer* and *Trichoderma harzianum* (Table 1).

Endophytic fungi possessing the ability to synthesize protease enzymes facilitated the expeditious screening of a substantial quantity of fungi through the utilization of primary and secondary methodologies. The evaluation of *Trichoderma harzianum* fungi was conducted via the implementation of silver nanoparticles, Fourier-transform infrared spectroscopy (FTIR), and gas chromatography-mass spectrometry (GC-MS) techniques, which enabled the identification of bioactive compounds derived from *Trichoderma harzianum*.

The sponge mats were subjected to treatment with varying concentrations of $AgNO^3$ and were subsequently incubated at room temperature in a dark environment. The alteration in coloration was observed within the mycelium of the fungi under investigation, transitioning from a light-yellow hue to a deep brown shade. Following the addition of an aqueous solution of $AgNO^3$ at a concentration of 1 mM, the fungi were subjected to optical measurements utilizing a UV-Vis spectrophotometer. This analysis revealed peak absorption at 410 nm. The synthesis of silver nanoparticles by the endophytic fungi *Trichoderma harzianum* occurred at concentrations of 0.5, 1.0, 1.5, and 2.0 µg/ml, yielding respective values of (1.12±0.09), (0.98±0.08), (0.90±0.01), and (0.85±0.00) as determined.

The alteration in hue was observed in both the fungal mat and the cell filtrate of the test fungi, resulting in a shift from a pale yellow to a dark brown coloration. Following the addition of aqueous $AgNO^3$ (1 mM), the fungal cell filtrate underwent

optical measurements via UV-Vis spectrophotometry. This analysis revealed an absorbance of the peak at 410-420 nm, which was indicative of the presence of silver nanoparticles [20]. The biosynthesis and optimization of silver nanoparticles were conducted using extracts from the endophytic fungi *Penicillium oxalium* strain LA-1, which was isolated from the medicinal plant *Limonia acidissima* [19]. In the current investigation, $AgNO^3$ was synthesized with a high degree of efficacy, with a concentration of 0.5 µg/ml yielding a result of 1.12 ± 0.09 (Table 2).

Table 1 Isolation and identification of endophytic fungi from marine environs

S. No.	Name of the endophytic fungi	*Acanthus ilicifolius*	*Aegiceras corniculatum*	*Vicennia marina*	*Ceriops decandra*	*Excoecaria agallocha*	*Rhizophora mucronata*
1.	*Aspergillus flavus*	5	2	3	6	6	-
2.	*A. fumigatus*	3	5	2	5	4	2
3.	*A. niger*	2	3	-	3	6	5
4.	*A. terreus*	5	4	3	-	8	6
5.	*A. ochraceous*	6	9	6	8	3	4
6.	*Chaetomiun* sp.	4	5	6	8	-	2
7.	*Fusarium* sp.	2	4	5	6	3	3
8.	*F. moniliforme*	6	3	-	2	5	6
9.	*F. oxysporum*	4	3	-	4	6	-
10.	*Pencillium chrysogenum*	8	5	4	5	4	3
11.	*P. citrinum*	6	3	3	6	3	-
12.	*P. janthinellum*	4	5	2	-	-	2
13.	*P. purpurrescens*	5	-	5	3	5	6
14.	*R. stolonifer*	4	4	6	5	2	4
15.	*Trichoderma harzianum*	3	2	4	4	-	5
Total number of colonies		67	57	49	65	55	48

Table 2 Biosynthesis of silver nanoparticle in *T. harzianum*

Different concentration Silver nitrate (µg/ml)	Quantity (µg/ml)
0.5	1.12 ± 0.09
1.0	0.98 ± 0.08
1.5	0.90 ± 0.01
2.0	0.85 ± 0.00

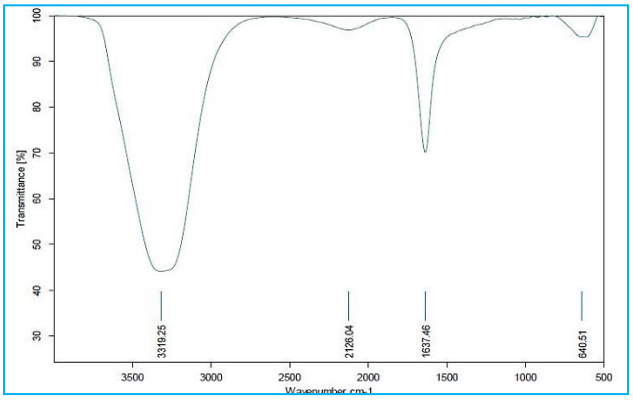

Fig 1 FTIR analysis for biosynthesis of silver nanoparticle in *Trichoderma harzianum*

Table 3 FTIR analysis for biosynthesis of silver nanoparticle in *Trichoderma harzianum*

Group frequency cm^{-1} of the sample	Functional group assignment
3319.25	Amines, Imines (=N–H); one bands
2126.04	Unsaturated Nitrogen Compounds, C≡N Stretching vibrations, isocyonides
1637.46	C–C Multiple bonds stretching, Alkene, disubstituted, *gem*
640.51	Halogen compounds, C–X Stretching vibrations C–CI

The structure of the synthesized $AgNO^3$ was characterized using Fourier transform infrared spectroscopy (FTIR) (Table 3). The analysis of the FTIR spectra revealed the presence of distinct peaks at 3319.25 cm^-1, indicating the presence of amines, imines (=N–H), and a single functional group. Another peak observed at 2126.04 cm^-1 suggested the presence of unsaturated nitrogen compounds, specifically C≡N stretching vibrations and isocyanides. Additionally, a peak at 1637.46 cm^-1 indicated the presence of C–C multiple bond stretching, suggesting the existence of an alkene and a disubstituted pearl. Lastly, the peak observed at 640.51 cm^-1 indicated the presence of halogen compounds and C–X stretching vibrations, specifically C–Cl bonds (Fig 3).

Fourier Transform Infrared (FTIR) measurements were conducted on cell filtrates, revealing the presence of distinct spikes at 3377, indicating the existence of free OH and NH groups. Additionally, a peak at 2139 was observed, suggesting the presence of an aromatic CH stretch. Furthermore, a peak at 1644 indicated the occurrence of C-C stretching, while a peak at 1965 confirmed the presence of dissatisfaction within a molecule [20]. Notably, a high intensity at 726 indicated the occurrence of C-Cl stretching. These findings align with a previous study conducted by Nirjanta, as reported by Devi *et al*. in 2013, which investigated the synthesis of silver nanoparticles using an endophytic fungus [22].

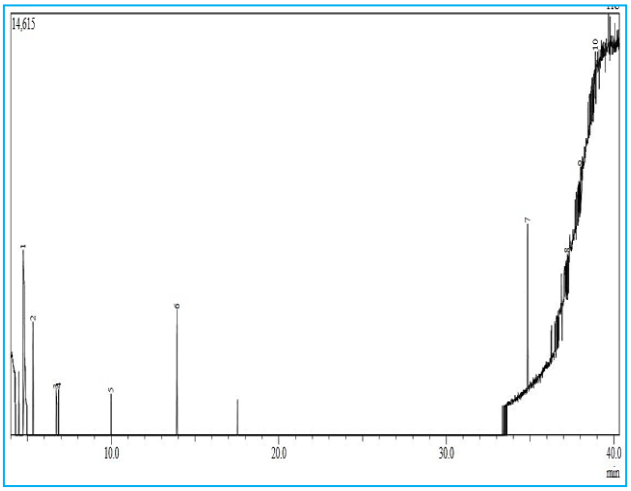

Fig 2 GC-MS analysis for biosynthesized of silver nanoparticle in *Trichoderma harzianum*

Table 4 List of identified bioactive compounds of silver nanoparticle extract of endophytic fungus *Trichoderma harzianum*

S. No.	Compound	Molecular formula	Molecular weight	Retention time	Peak area %
1.	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	180	4.742	33.25
2.	1-Pyrrolidinecarboxamide, N-1,3-Hexadienyl-	$C_{11}H_{18}N_2O$	194	5.329	9.92
3.	2-Keto-Butyric-Acid	$C_4H_6O_3$	102	6.705	6.9
4.	2-Keto-Butyric-Acid	$C_4H_6O_3$	102	6.859	2.92
5.	(SS)- or (RR)-2,3-hexanediol	$C_6H_{14}O_2$	118	9.99	2.62
6.	2,3,4,4-Tretrapropyl-1-(Trimethylsilyl)-1-(Trimethylsilyloxy)-1,3-Diaza-2,4-Diborabutane	$C_{18}H_{44}B_2N_2OSi_2$	382	13.918	9.87
7.	1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester	$C_{20}H_{26}O_4$	390	34.852	16.99
8.	3,4-Dihydro-4-(1,3-Dioxolan-2-yl)-5,7-Dimethoxy-1(2h)-Benzopyran-2-One	$C_{14}H_{16}O_6$	280	37.213	5.38
9.	6A.Alpha. 12A.Alpha. 5'.Beta.(CIS-.Alpha.,.Alpha.)-12A-Hydroxyrotenoide	$C_{23}H_{22}O_8$	426	38.015	6.78
10.	(1.Alpha. 4. Alpha., 4A.Alpha. 10A.Beta.)-1,4,4A,5,6,7,8,9,10,10A-Decahydro-'1,4,11,11-Tetramethyl-1,4-Methanocycloocta[D]Pyridaz	$C_{15}H_{26}N_2$	234	38.898	5.38

The GC-MS chromatogram of the silver nanoparticle extract obtained from the endophytic fungus *Trichoderma harzianum* revealed the presence of distinct peaks corresponding to various compounds (Fig 2). The identification of these bioactive compounds was carried out based on their molecular formula, peak area, and retention time. To compare the mass spectral analysis of these components, the NIST library was utilized.

Among the identified compounds, dl-Glyceraldehyde dimer ($C_6H_{12}O_6$) exhibited a peak area of 33.25 and a retention time of 4.742. Another compound, 1-Pyrrolidinecarboxamide, N-1,3-Hexadienyl- ($C_{11}H_{18}N_2O$), displayed a peak area of 9.92 and a retention time of 5.329. Additionally, 2-Keto-Butyric-Acid ($C_4H_6O_3$) was observed with a peak area of 6.9 and a retention time of 6.705. This same compound, 2-Keto-Butyric-Acid ($C_4H_6O_3$), was also detected with a peak area of 2.92 and a retention time of 6.859.

Furthermore, (SS) or (RR)-2,3-hexanediol (C_6H14O2) exhibited a peak area of 2.62 and a retention time of 2.62. Another compound, 2, 3, 4, 4-Tretrapropyl-1-(Trimethylsilyl)-1-(Trimethylsilyloxy)-1,3-Diaza-2,4-Diborabutane ($C_{18}H_{44}B_2N_2OSi_2$), displayed a peak area of 9.87 and a retention time of 13.918. Moreover, 1,2-Benzenedicarboxylic Acid, Dicyclohexyl Ester (C20H26O4) was observed with a peak area of 16.99 and a retention time of 34.852. Additionally, 3,4-Dihydro-4-(1,3-Dioxolan-2-yl)-5,7-Dimethoxy-1(2h)-Benzopyran-2-One (C14H16O6) exhibited a peak area of 5.38 and a retention time of 37.213 (Table 4).

Another compound, *Colletotrichum gloeosporioides*, which is an endophytic fungus obtained from the medicinal plant *Phlogacanthus thyrsiflorus*, was found to produce volatile compounds from its crude extract. These compounds were then analyzed using GC-MS analysis. The main components identified in the analysis were phenol, 2,4-bis(1,1-dimethylethyl), 1-hexadecene, 1-hexadecanol, hexadecanoic acid, octadecanoic acid methyl ether, and 1-nonadecene [23]. The presence of these metabolites suggests that the endophytic *Colletotrichum gloeosporioides* has the ability to produce bioactive compounds. Furthermore, the crude ethyl acetate extract of the endophyte *Colletotrichum gloeosporioides* isolated from *Lannea corammendalica* was also subjected to GC-MS analysis. This analysis revealed the presence of important compounds such as 9-octadecanamide, hexadecananamide, diethylpyrthalate, 2-methyl-3-methyl-3-hexene, and 3-ethyl-2, 4-dimethylpentane, which exhibited antimicrobial properties [24].

CONCLUSION

Conclusively, this study was conducted in the study area of Mallipattinam and Kollukadu in the Pattukkottai taluk of Thanjavur district, Tamil Nadu, India. The main objective of this study was to collect various medicinal plant species, including *Acanthus ilicifolius*, *Aegiceras corniculatum*, *Avicennia marina*, *Ceriops decandra*, *Excoecaria agallocha* and *Rhizophora mucronata*. A total of 16 endophytic fungi were isolated and identified from these plant species. It was found that the minimum number of fungi was isolated from the *Rhizophora mucronata* plant. The screening of a large number of fungi was facilitated by the presence of endophytic fungi that possess the ability to synthesize protease enzymes. This screening process involved the utilization of primary and secondary methodologies. Furthermore, the evaluation of *Trichoderma harzianum* fungi was conducted using silver nanoparticles, Fourier-transform infrared spectroscopy (FTIR), and gas chromatography-mass spectrometry (GC-MS) techniques. These techniques enabled the identification of bioactive compounds derived from *Trichoderma harzianum*. In this study, $AgNO_3$ was synthesized with a high degree of efficacy, and a concentration of 0.5 µg/ml resulted in a yield of 1.12 ± 0.09. Additionally, the presence of halogen compounds and C–X stretching vibrations, specifically C–Cl bonds, was indicated by the peak observed at 640.51 cm^-1. The GC-MS chromatogram of the silver nanoparticle extract obtained from the endophytic fungus *Trichoderma harzianum* revealed the presence of 10 bioactive compounds.

LITERATURE CITED

1. Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. 2013. Prospecting endophytic fungal assemblage of *digitalis lanata* Ehrh. (Foxglove) as a novel source of digoxin: a cardiac glycoside. 3 *Biotech* 3(4): 335-340.
2. Strobel G, Daisy B. 2013. Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Review* 67(4): 491-502.
3. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S, Jones M, Lazarides L, Steadman VA, Cohen DR, Felix CR, Fetterman KA, Millett WP, Nitti AG, Zullo AM, Chen C, Lewis K. 2015. A new antibiotic kills pathogens without detectable resistance. *Nature* 517(7535): 455-459.

4. Strobel G, Daisy B, Castillo U, Harper J. 2004. Natural products from endophytic microorganisms. *Journal of Nat. Prod*. 67: 257-268.
5. Aly AH, Debbab A, Kjer J, Proksch P. 2010. Fungal endophytes from higher plants: A prolific source of phytochemicals and other bioactive natural products. *Fungal Divers* 41: 1-16.
6. Prabukumar S, Sathishkumar G, Rajkuberan C, Gobinath C, Asad S, Hodhod MS, Fuad A, Sivaramakrishnan S. 2018. Isolation of limonoid compound (Hamisonine) from endophytic fungi *Penicillium oxalicum* LA-1 (KX622790) of *Limonia acidissima* L. for its larvicidal efficacy against LF vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Environ. Sci. Pollution Research* 24: 21272-21282.
7. Sardul S, Harshita S, Shyamji S. 2017. Biosynthesis of silver nanoparticles by endophytic fungi: Its mechanism, characterization techniques and antimicrobial potential. *African Journal of Biotechnology* 16(4): 684-698.
8. Manjunatha D, Akarsh S, Nandish G, Sowmya HV, Megha GT, Nagaraju S, Thippeswamy B. 2022. Eco-friendly synthesized silver nanoparticles from endophytic fungus *Phyllosticta owaniana* – KUMBMDBT-32 and evaluation of biomedical Properties. *Research Square* 1-16. https://doi.org/10.21203/rs.3.rs-2345744/v1
9. Spiteller P. 2015. Chemical ecology of fungi. *Nat. Prod. Rep.* 32(7): 971-993.
10. Harris MI, Hadden WC, Knowler WC, Bennett PH. 1987. Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20-74 yr. *Diabetes* 36(4): 523-534.
11. Zimmet P, Alberti KG, Shaw J. 2001. Global and societal implications of the diabetes epidemic. *Nature* 414(6865): 782-787.
12. Huang WY, Cai YZ, Xing J, Corke H, Sun M. 2007. A potential antioxidant resource: endophytic fungi isolated from traditional Chinese medicinal plants. *Economic Botany* 6: 14-30.
13. Larran S, Perelló A, Simón MR, Moreno V. 2007. The endophytic fungi from wheat (*Triticum aestivum* L.). *World Journal of Microbiology and Biotechnology* ZS: 565-572.
14.Raper KB, Fennell DI. 1965. The genus *Aspergillus*. Williams and Wilkins Co, Baltimore, Maryland. pp 686.
15. Gillman JC. 1957. A manual of soil fungi. Revised 2nd Edition, Oxford and IBH publishing company (Indian reprint) Calcutta, Bombay, New Delhi.
16. Smith G. 1946. A manual of the *Aspergilli. Nature* 157: 462.
17. Subramanian CV. 1971. Hypomycetes. New Delhi: ICAR Publication.
18. Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. pp 608.
19. Prabu Kumar S, Rajkuberan C, Rajiv P, Sathishkumar G, Sivaramakrishnan S, Kamel A, Martin Vand Kamil K. 2021. Functional attributes of myco-synthesized silver nanoparticles from endophytic fungi: A new implication in biomedical applications. *Biomedical Applications Biology* 10: 473: 1-20. https://doi.org/10.3390/biology10060473.
20. Vardhana J, Kathiravan G, Magesh CR. 2019. Biosynthesis of silver nanoparticles from endophytic fungi, and its cytotoxic activity. *Bio Nano Science*. pp 1-8. https://doi.org/10.1007/s12668-019-00631-1
21. Srinivasan K, Sivasubramanian S, Kumaravel S. 2013. Phytochemical profiling and GC-MS study of *Adhatoda vasica* leaves. *Int. Jr. Pharm. Bio. Science* 5(1): 714-720.
22. Nameirakpam ND, Dheeban SP, Sutha S. 2012. Biomimetic synthesis of silver nanoparticles from an endophytic fungus and their antimicrobial efficacy. *International Journal of Biomedical and Advance Research* 3(5): 409-415.
23. Devi NN, Singh MS. 2013. GC-MS analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* Nees. *Int. Jr. Pharm. Sci. Rev. Research* 23(2): 392-395.
24. Premjanu N, Jaynthy C. 2015. Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Colletotrichum gloeosporioides* isolated from *Lannea corammendalica*. *International Journal of Chem. Tech. Research* 7: 369-374.