International Journal of Research and Review
Volume 11; Issue: 8; August 2024
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Isolation, Screening and Biocidal Activity of Secondary Metabolites from Fungi of Paddy Field Soil Samples in Selected Alanganallur Sub Regions, Madurai, Tamil Nadu

R. Seenivasan[1], M. Krishnamoorthy[2], P. Velladurai[3]

[1,2,3]PG & Research Department of Zoology, The American College, Madurai, Tamil Nadu, India.625002

Corresponding Author: P. Velladurai

DOI: https://doi.org/10.52403/ijrr.20240835

ABSTRACT

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. The soil serves as a reservoir for many microbial communities of plants and herbs which are producing, CO_2 and nitrogen. Paddy (*Oryza sativa* L.) is the most important cereal crop of the world. Paddy field soil contains rich organic matters like old stubble, paddy straw, senescent roots and wastes. It also contains numerous types of fungi, which support rice production as well as maintain the fertility of paddy soil. This study investigates the secondary metabolites produced by various fungal species isolated from the Alanganallur region, aiming to uncover novel bioactive compounds with potential pharmaceutical and agricultural applications. Soil samples were systematically collected and cultured to isolate diverse fungal strains. The secondary metabolites were extracted using solvent extraction methods and subsequently analyzed using microbial and spectroscopic techniques, including FT-IR spectroscopy. The screening revealed a wide array of secondary metabolite compounds which exhibited significant antibacterial activity when subjected to antibacterial tests. Notably, a subset of these metabolites demonstrated potent activity against *Staphylococcus aureus* and *E. coli*, highlighting their potential as leads for new antibacterial agents. This research underscores the rich biodiversity of soil fungi in the Alanganallur region and their capacity to produce bioactive secondary metabolites. The findings suggest that these metabolites could serve as a valuable resource for the development of novel therapeutic agents and biopesticides.

Keywords: Metabolites, Nitrogen, Soil fungi, Antibacterial

INTRODUCTION

Soil is a vital resource for agricultural production, food security, and life processes.[1] It contains microbial activity, playing a crucial role in decomposition and nutrient cycling. Soil microbial diversity is essential for successful microbial bioinoculants and soil health.[2] These microorganisms significantly impact soil ecosystems by decomposing organic matter, recycling nutrients, and controlling biological processes. Soil is an oligotrophic medium for fungal growth, as they are limited and present for short periods.[3]

Fungi are dormant or slow-metabolizing, distributing organic matter away from roots. The concentration of microbes is highest near the roots and arbuscular mycorrhizal fungi, where exudates are a significant source of organic energy. Fungi are more closely related to animals than plants, with 80 percent or more of the same genes as humans. [**4**] Rice, the world's most important staple food crop, is crucial for national food security and should be given special status among cereals. However, increasing rice cultivation sustainably with reduced inputs and less exploitation of natural resources is a challenge for rice-growing countries worldwide. Rice production is only exceeded by wheat, and with the increasing global population, it is essential to augment cereal production, mainly wheat, rice, and maize, which account for half of human caloric intake.[**5**] Chemical fertilizers, such as nitrogen and phosphorous, are used to enhance grain yield, but their increased use has led to problems such as water contamination, soil degradation, and loss of biodiversity, ultimately posing health risks for humans.[**6**] Paddy field soil contains rich organic matter, such as old stubble, paddy straw, senescent roots, and wastes. Soil microorganisms play a vital role in metabolic reactions, such as mineralization of soil organic nitrogen and decomposition of rice straw and compost, which support rice production and maintain soil fertility.[**7**] The physico-chemical properties of agricultural soil play a crucial role in agricultural production and forest development. Soil quality analysis encompasses parameters such as pH, texture, moisture, electrical conductivity, and nitrogen content, all of which impact the soil's ability to operate as part of a healthy ecosystem.[**8**] pH levels determine soil acidity, with ranges below 6 considered acidic, 6-8.5 as normal, and above 8.5 as alkaline. Soil texture, influenced by particle size, affects aeration, root penetration, and nutritional status. Moisture content influences nutrient absorption and soil texture. Electrical conductivity measures soil quality by assessing ion concentration in the soil solution. Furthermore, nitrogen, a critical plant nutrient, plays a key role in plant growth and exists in various forms within the soil and water systems.[**9**] Phosphorus is a crucial element for plant growth, present in every living cell. It acts as an energy storage and is essential for plant development. Additionally, soil organic matter is valuable for agricultural practices; its absence can negatively impact soil quality, while its presence enhances soil processes [**10**] Potassium is also important for various physiological processes in plants, such as metabolic reactions and regulation of photosynthesis [**11**]. Adding organic matter to the soil in the form of manures or compost is beneficial. Furthermore, the decrease in soil organic matter content from surface to subsoil is a concern due to levelling.

MATERIALS & METHODS

i) Study site and location

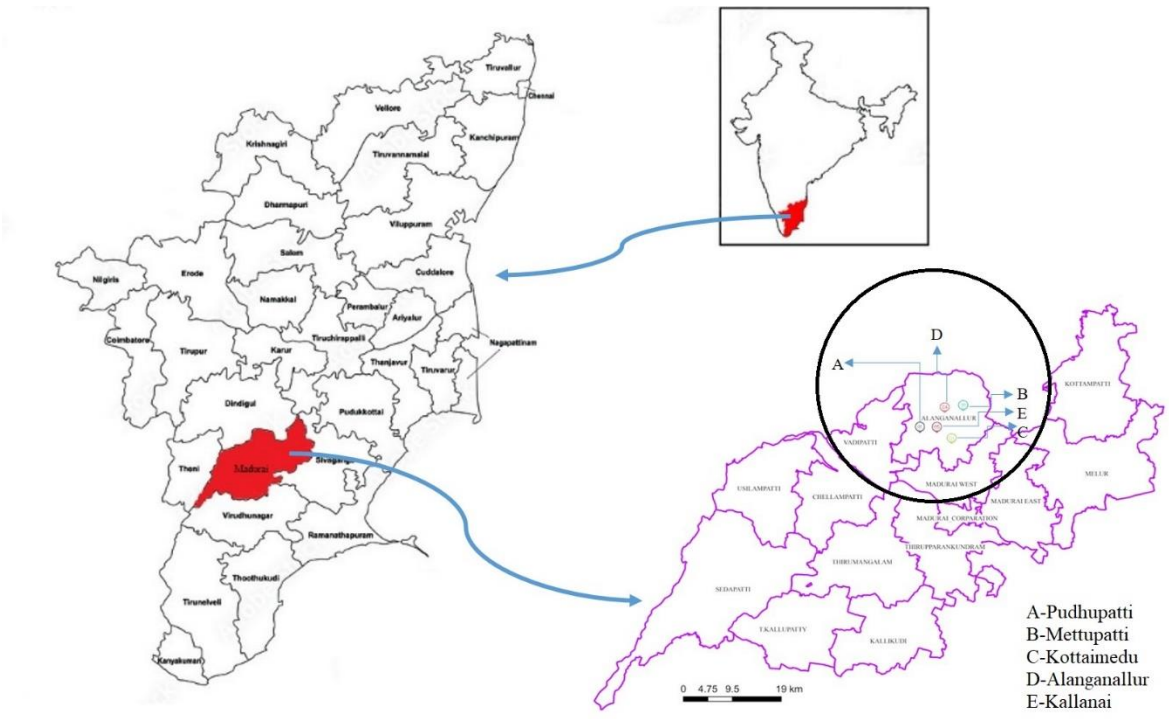
Alanganallur is a panchayat town in Madurai district in the State of Tamil Nadu, India. Agriculture is carried out in large area around this place. Paddy, Sugar cane, Coconut and Plantain are the major crops. It is located between latitude of 10°2'52.05"N and longitude of 78°5'21.98"E.

Collection of soil samples

The soil samples were collected from five different Paddy fields at five different locations in and around Alanganallur, Madurai District. The soil samples were collected from different Paddy fields (up to 15cm depth) into small sterilized polythene bags brought to laboratory stored at 4^{o}C for further studies. The collected soil samples were from A. Pudhupatti, Mettupatti, Kottaimedu, Alanganallur and Kallanai fields.

Chemicals and Media

All Culture media components and Chemicals used in this study such as Nutrient Agar, Potato Dextrose Agar, Actinomycete Isolation Agar (AIA), Muller Hinton Agar, Potato Dextrose Broth, Nutrient Broth, Nalidixic acid, and Cycloheximide were of analytical grade and purchased from Hi-media laboratory Pvt. Ltd, Mumbai, India. The accurate amount was weighed and autoclaved at 121^{o}C for 30 minutes.



Map showing the sample collection sites in an around Alanganallur town, Madurai, Tamilnadu, India

Figure. 1 Map showing the sample collection sites in an around Alanganallur town, Madurai, Tamil Nadu, India.

Physico-Chemical characteristics of soil samples

The collected soil was characterized for its physico-chemical properties. The physico-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH, Electrical Conductivity, Nitrogen, Phosphorus, Potassium, Iron, Manganese, Zinc, and Copper were analyzed. The physico-chemical parameters of the soil samples were analyzed at Soil Testing Laboratory, Department of Agriculture, Government of Tamil Nadu, Madurai.

Isolation of soil fungi

Soil Dilution Plate Method (Waksman, 1922): Soil dilutions were made by suspending 1g of soil of each sample in 10ml of sterile distilled water. Dilutions of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing sterile Potato Dextrose Agar medium. 1% streptomycin solution was added to the medium for preventing bacterial growth, before pouring into Petri

plates. The plates were then incubated at 26° C to 28° C for 4-7 days. After 4-7 days of incubation, the colonies growing on Potato Dextrose Agar plates, with different morphology, were counted and purified on medium separately. Organisms were easily isolated because they formed surface colonies that were well dispersed particularly at higher dilutions.

Identification of soil fungi

The fungi were identified with the help of literature (Nagamani Kunwar and Manoharachary, 2014). Then the identified colonies were transferred to Petri dishes containing agar. Identification of the organism was made with the help of the relevant literature (Thom & Raper 1941).

Extraction of secondary metabolites from Isolated Fungi

Extraction of secondary metabolites were carried out with slight modification from literature (Sadrati,2013). After fermentation, culture broth was separated from the mycelia by filtration. Then the separated broth was mixed with an equal volume of ethyl acetate (100%). And the mixture was vigorously mixed by vortex to extract extracellular secondary metabolites which is present in broth. The secondary metabolites were released inorganic phase of the mixture. The organic phase was removed carefully. Then the separated mycelium was blended using mortar and pestle by adding small volume of ethyl acetate which is used to breakdown the cell wall to release intracellular metabolites, and then the metabolites were separated by filtration. Both the organic phase and filtrate were mixed and kept for rotary evaporation. After evaporation secondary metabolites were collected in eppendorf tube and weighed.

Antibiotic sensitivity test

Muller Hinton agar plates were prepared and isolated organisms were swabbed on the plates using a sterile cotton swab. After 15 minutes, antibiotic disc was placed on the plates using sterile forceps in such a way that the distance between two discs were at least 20mm. The plates were incubated at 37°C for 24 hours. The presence and size of inhibitory zones were observed.

Anti-fungal activity (Disc diffusion method)

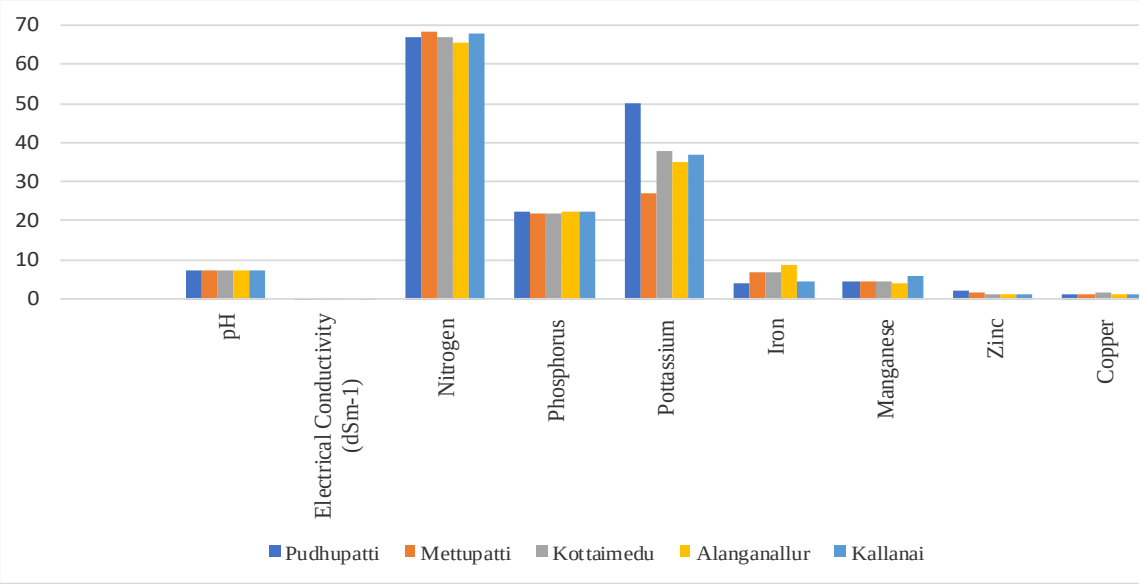
Antibacterial activity of isolated fungal extracts was done using Disc diffusion Method. Freshly prepared 24hrs overnight culture of isolated fungi were swabbed over the Muller Hinton Agar (MHA) plate. Commercially available Fluconazole disc were placed on respective plates. The plates were incubated at 37°C for 24hrs. After incubation zone of inhibition was observed and measured.

FT – IR Spectrometry (Fourier Transform – Infra Red spectrum analysis)

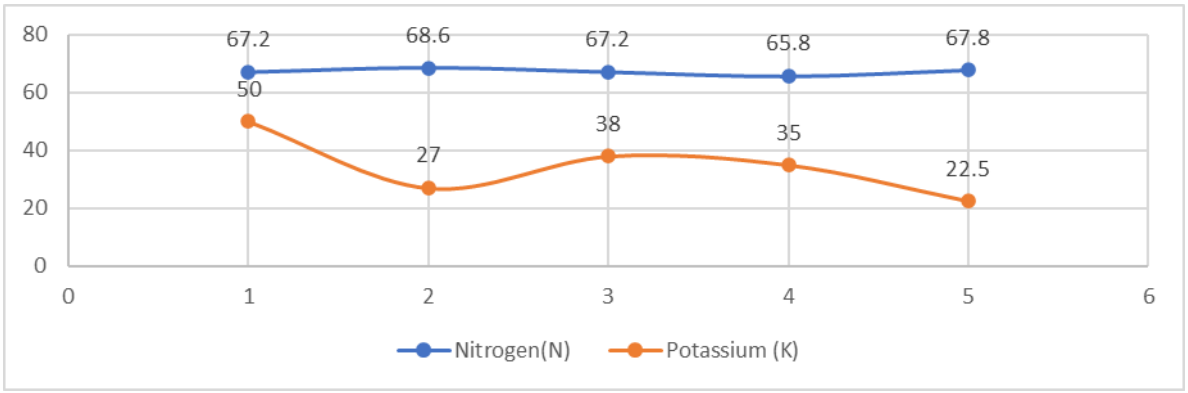
The fungal extract samples (10mg) were mixed with 100mg of dried Potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectrometrically. The frequencies of different components present in active sample were analyzed. (Devi, *et al.*, 2010).

RESULTS

The values of all physico-chemical characteristics were plotted in a Graph .1. Among the selected physico-chemical parameters, Nitrogen was observed as a predominant element present in the soils of the all-study sites. Among study sites, Mettupatti was recorded for high Nitrogen and Potassium rich soil condition. Followed by the results from the graph.2 indicates that Nitrogen is a predominant element in all study areas followed by Potassium. During the study, very low electrical conductivity was observed in all 5 study sites. In terms of Microelements, Iron showed the maximum level and Zinc observed in minimum quantity.

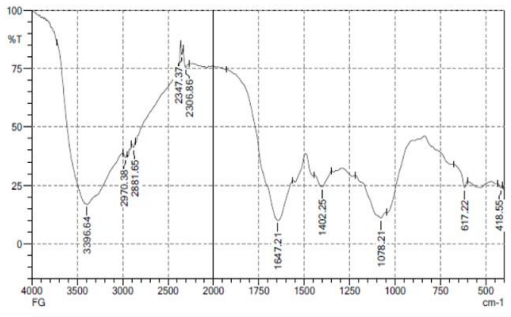


Graph.1 Physico-chemical characteristics of collected samples from different regions.



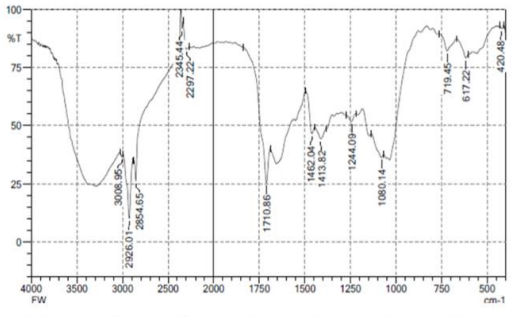
Graph .2 Occurrence of N and K (mg/kg) from different study sites (1- A.Pudhupatti, 2- Mettupatti, 3- Kottaimedu, 4- Alanganallur, 5- Kallanai)

FT-IR spectra showed the presence of characteristic transmittance bands of hydroxyl groups (-OH), amines (-NH_2), alkyl (-C-C), carbonyl (-CO), carboxylic acids (-COOH). In *Aspergillus flavus* species showed highest peak at 3396.64 cm^{-1} which indicates N-H amide group. The transmittance band at 2970.38 cm^{-1} depicts C-H stretching. 2881.65 cm^{-1} band shows O-H stretching, mostly it indicates the presence of glucose. The C=C, C=N stretching groups was seen in the band at 2347.37 cm^{-1}. The peaks 2306.86 cm^{-1}, 1647.21 cm^{-1}, 1402.25 cm^{-1}, 1078.21 cm^{-1} shows the C=O, C=N or C-OH stretching. The C-Cl alkyl seen in the 671.22 cm^{-1} band. The finger print transmittance bands are difficult to interpret such as 418.55 cm^{-1}. *Aspergillus fumigatus* species showed highest transmittance band at 3008.95 which indicates =C-H stretching groups. The C-H stretching was seen in the following bands at 2926.01 cm^{-1}, 2854.65 cm^{-1}. 2345.44 cm^{-1} transmittance bond indicates either C=C or N=N stretching.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	418.55	24.04	1.32	437.84	410.84	2027.773	16.815
2	617.22	24.06	4.35	677.01	601.79	5283.453	56.652
3	1078.21	10.99	5.45	1220.94	1045.42	14244.481	446.343
4	1402.25	24.41	5.83	1444.68	1350.17	6877.880	293.284
5	1647.21	10.00	27.90	1930.74	1564.27	21062.353	3076.343
6	2306.86	75.41	5.35	2335.80	2268.29	1528.533	198.459
7	2347.37	80.91	3.66	2360.87	2335.80	435.316	50.327
8	2881.65	41.21	2.01	2900.94	2860.43	2341.959	42.979
9	2970.38	36.91	1.63	2997.38	2953.02	2759.045	35.708
10	3396.64	16.83	48.17	3720.69	2997.38	47803.135	20710.945

1. FT-IR Analysis of secondary metabolites of *Aspergillus flavus*

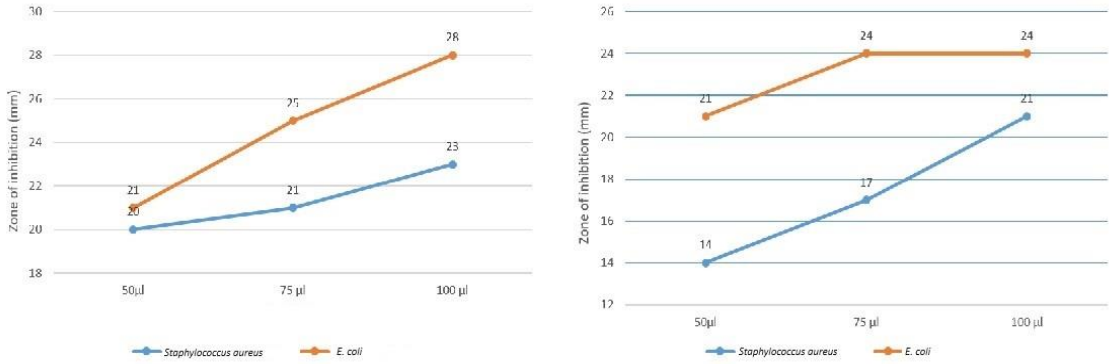


No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	420.48	91.64	1.64	432.05	410.84	163.289	20.552
2	617.22	79.11	2.96	667.37	603.72	1081.944	61.694
3	719.45	82.06	6.37	763.81	667.37	1378.253	254.302
4	1080.14	36.32	2.79	1136.07	1068.56	4031.138	117.716
5	1244.09	51.71	3.15	1274.95	1220.94	2515.683	75.358
6	1413.82	44.32	4.72	1444.68	1382.96	3293.093	147.902
7	1462.04	46.57	8.07	1496.76	1444.68	2377.034	150.753
8	1710.86	25.30	21.05	1838.16	1689.64	5843.271	185.384
9	2297.22	82.86	5.98	2335.80	2266.36	1007.317	275.819
10	2345.44	91.58	5.36	2360.87	2335.80	137.134	74.824
11	2854.65	26.14	10.61	2873.94	2395.59	17804.099	-2624.513
12	2926.01	10.00	26.05	2993.52	2883.58	8155.822	1163.690
13	3008.95	36.50	1.74	3028.24	2993.52	2170.886	28.011

2. FT-IR Analysis of secondary metabolites of *Aspergillus fumigatus*

Figure.2: Results of FT-IR Analysis showing the peaks related to isolated secondary metabolites from 2 different fungi. (1-*Aspergillus flavus*, 2-*Aspergillus fumigatus*)

The C=N stretching was seen in the 2297.22 cm^{-1} and 1710.86 cm^{-1}. The CH_2 bonding vibration was seen in the following transmittance peaks includes 1462.04 cm^{-1}, 1413.82 cm^{-1}, 1244.09 cm^{-1} and 1080.14 cm^{-1}. The C-Cl alkyl seen in the 719.45 cm^{-1} and 617.22 cm^{-1} band. The finger print transmittance bands are difficult to interpret such as 420.48 cm^{-1}.



Graph. 3 Results from the antimicrobial activity from secondary metabolites isolated from 2 different fungi tested against 2 different test organisms. (A-*Aspergillus flavus*, B-*Aspergillus fumigatus*)

The antimicrobial activity of secondary metabolites was evaluated against microbial strains, including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli*). The disc diffusion method was employed to determine the inhibitory effect of the test substance. The results from the antimicrobial activity showed that *E. coli* obtained higher zone of inhibition against *Aspergillus flavus* (28mm), compared to *Staphylococcus aureus.* Similar trend was recorded also from the antimicrobial activity using *Aspergillus fumigatus.* The zones of inhibition (in mm) observed for each microbial strain are summarized in Graph. 3. The Secondary metabolites exhibited significant inhibitory activity against *Staphylococcus aureus* and *E. coli* with the largest zone of inhibition observed against *Aspergillus fumigatus*.

DISCUSSION

From the above results, agriculture soil is a dynamic medium in which a large number

of pathogenic and non-pathogenic bacterial and fungal flora live in close association. Microbes in the soil are the key to carbon and nitrogen recycling. In our present study, five different agricultural soil samples were collected and obtained cultures were purified and screened for fungal population. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content and moisture. The results of soil pH and soil texture were determined the fungal population and their diversity in agricultural field (Wear, 1962). The soil pH organic content and water are the main factors affecting the fugal population and diversity. The organic carbon, nitrogen, phosphate, potassium are important for fungi and the obtained results highly aligned with the results of George et.al 1995, Dellagi et.al 2020. According to Haro et.al 2019, soil-dwelling microorganisms associated with plant roots may improve K^+ nutrition in plants via three different effects, especially when plants grow in lands affected by K^+ deprivation. Using these levels of $K^{+,}$ soil fungus used to fertilize the paddy plants from the study sites. In the present study 2 different species of fungi show the predominance from the five different agricultural fields. *Aspergillus flavus, Aspergillus fumigatus* was subjected to antibacterial susceptibility test was done against commercial antibiotics. Similar to the findings of Bhardwaj, 2017, the fungus showed good zone of inhibition. When the concentration of disc increased the zone of inhibition also increased. (Table-3). Similar to the observations of Hateet et.al 2014, Rodrigues et.al 2000, From the results of antimicrobial susceptibility tests, in A, there is a gradual increase in zone of inhibition, while increasing the concentration of metabolite. The comparative results of antimicrobial susceptibility tests showed the zone of inhibition was maximum from the metabolites of *Aspergillus flavus* against *Staphylococcus aureus* compared to *E. coli*. (Mustapha et.al., 2019, Furtado et.al., 2005). It is suggested that further purification and characterization of the secondary metabolites from *Aspergillus flavus* will have a promising effect against *staphylococcus aureus* and related infections. Zone of inhibition (mm) obtained from *E. coli* also displayed a moderate resistance against secondary metabolites from *Aspergillus flavus* and *Aspergillus fumigatus.*

CONCLUSION

The study reveals a vast array of bioactive compounds in soil fungi, including antimicrobial, antifungal, anticancer, and anti-inflammatory properties. These compounds have strong activity in preliminary tests, suggesting potential for pharmaceutical and agrochemical development. The study emphasizes the importance of exploring microbial diversity in soil ecosystems for discovering new bioactive compounds. Future research should focus on optimizing culture conditions, understanding molecular mechanisms, and assessing safety and efficacy in *in vivo* models.

Declaration by Authors

All the authors undersigned and hereby declare that this is an original work and best of their knowledge this research work/draft contains no material previously published by any other person except where due acknowledgement has been made.

Acknowledgement: The Authors gratefully acknowledge the support and contributions of research department of Zoology and The American College that made this research possible. Authors were indebted to the PG Zoology department which provided the necessary resources to undertake this research. Additionally, Authors would like to thank colleagues at the International

Research Centre, Kalasalingam Academy of Research & Education, Krishnankoil for their collaborative spirit and constructive feedback during the course of this work.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Pozza, L. E., & Field, D. J. (2020). The science of soil security and food security. *Soil Security*, *1*, 100002.
2. Bertola, M., Ferrarini, A., & Visioli, G. (2021). Improvement of Soil Microbial Diversity through Sustainable Agricultural Practices and Its Evaluation by -Omics Approaches: A Perspective for the Environment, Food Quality and Human Safety. *Microorganisms*, *9*(7), 1400. https://doi.org/10.3390/microorganisms9071400
3. JOHN C. ZAK, HOWARD G. WILDMAN (2004) Biodiversity of Fungi
4. Khaliq, Abdul, Shaista Perveen, Khalid H. Alamer, Muhammad Zia Ul Haq, Zaiba Rafique, Ibtisam M. Alsudays, Ashwaq T. Althobaiti, Muneera A. Saleh, Saddam Hussain, and Houneida Attia. 2022. "Arbuscular Mycorrhizal Fungi Symbiosis to Enhance Plant–Soil Interaction" *Sustainability* 14, no. 13: 7840. https://doi.org/10.3390/su14137840
5. Erenstein, O., Jaleta, M., Sonder, K. *et al.* Global maize production, consumption and trade: trends and R&D implications. *Food Sec.* 14, 1295–1319 (2022). https://doi.org/10.1007/s12571-022-01288-7
6. Chandini & Kumar, Randeep & Kumar, Ravendra & Prakash, Om. (2019). The Impact of Chemical Fertilizers on our Environment and Ecosystem.
7. He C, Li K, Li J, Fan P, Ruan Y and Jia Z (2024) Rice straw increases microbial nitrogen fixation, bacterial and nifH genes abundance with the change of land use types. Front. Microbiol. 14:1283675. doi: 10.3389/fmicb.2023.1283675
8. Emmanuelle Garrigues, Michael S. Corson, Denis A. Angers, Hayo M.G. van der Werf, Christian Walter (2012) Soil quality in Life Cycle Assessment: Towards development of an indicator, Ecological Indicators, Volume 18, Pages 434-442, ISSN 1470-160X, https://doi.org/10.1016/j.ecolind.2011.12.014.
9. 沙利文, Shah Jahan & Wahocho, Niaz & Laghari, Ghulam & Laghari, Abdul & Banbhan, Ghulam Mustafa & HussainTalpur, Khalid & Ahmed, Tofique & Wahocho, Safdar & Lashari, Ayaz. (2016). Role of Nitrogen for Plant Growth and Development: A review. Advances in Environmental Biology. 10. 209-218.
10. Tale, K. S., & Ingole, S. (2015). A review on role of physico-chemical properties in soil quality. *Chemical Science Review and Letters*, *4*, 57-66.
11. Sharma, B. (2018). Chemical analysis of soil of pandoga sub watershed with the seasons in Una (HP), India. *IJRR*, *5*(10), 22-27.
12. Wear, J. I., & Patterson, R. M. (1962). Effect of soil pH and texture on the availability of water-soluble boron in the soil. *Soil Science Society of America Journal*, *26*(4), 344-346.
13. Bhardwaj, A. N. U. P. A. M. A., Chaman, S. O. N. I. A., & Verma, S. A. N. G. E. E. T. A. (2017). Production of antibacterial agent from fungi isolated from pharmaceutical soil sample by fermentation under optimized conditions. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(7), 110-115.
14. Waksman, S. A. (1922). A method for counting the number of fungi in the soil. *Journal of bacteriology*, *7*(3), 339-341.
15. Manohrachary, C., Nagaraju, D., Kunwar, I. K., & Nagamani, A. (2014). Soil fungi from Andhra Pradesh, India. *The Journal of Indian Botanical Society*, *93*(1and2), 16-34.
16. Thom, C., & Raper, K. B. (1941). *The Aspergillus glaucus group* (Vol. 424). US Department of Agriculture.
17. Sadrati, N., Daoud, H., Zerroug, A., Dahamna, S., & Bouharati, S. (2013). Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Triticum durum*). *Journal of plant protection research*, *53*(2).
18. George, E., Marschner, H., & Jakobsen, I. (1995). Role of Arbuscular Mycorrhizal Fungi in Uptake of Phosphorus and Nitrogen From Soil. *Critical Reviews in Biotechnology*, *15*(3–4), 257–270. https://doi.org/10.3109/07388559509147412

19. Dellagi, A., Quillere, I., & Hirel, B. (2020). Beneficial soil-borne bacteria and fungi: a promising way to improve plant nitrogen acquisition. *Journal of Experimental Botany*, *71*(15), 4469-4479.
20. Haro, Rosario, and Begoña Benito. 2019. "The Role of Soil Fungi in K^+ Plant Nutrition" *International Journal of Molecular Sciences* 20, no. 13: 3169. https://doi.org/10.3390/ijms20133169
21. Hateet, R. R., Muhsin, T. M., & Humadi, K. J. (2014). Antibacterial activities secondary metabolites from endophytic fungus *Fusarium solani*. *Journal of Basrah Researches (Sciences)*, *40*(1), 94-101.
22. Rodrigues, K. F., Hesse, M., & Werner, C. (2000). Antimicrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin*. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, *40*(4), 261-267.
23. Mustapha, F. A., Jai, J., Raikhan, N. N., Sharif, Z. I. M., & Yusof, N. M. (2019). Response surface methodology analysis towards biodegradability and antimicrobial activity of biopolymer film containing turmeric oil against *Aspergillus niger*. *Food Control*, *99*, 106-113.
24. Furtado, N. A. J. C., Fonseca, M. J. V., & Bastos, J. K. (2005). The potential of an *Aspergillus fumigatus* Brazilian strain to produce antimicrobial secondary metabolites. *Brazilian Journal of Microbiology*, *36*, 357-362.

How to cite this article: R. Seenivasan, M. Krishnamoorthy, P. Velladurai. Isolation, Screening and biocidal activity of secondary metabolites from fungi of paddy field soil samples in selected Alanganallur Sub Regions, Madurai, Tamil Nadu. *International Journal of Research and Review*. 2024; 11(8): 332-340. DOI: *https://doi.org/10.52403/ijrr.20240835*

******