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Research Article

Mycofloral Pattern Against Pathogens

Mycofloral Pattern and Its Insights of Bioactive Compounds Against Pathogens

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Abstract: Soil is considered a well-studied ecological niche for microorganisms that produce beneficial physiologically active natural compounds suited for medicinal purposes. The current study sought to elucidate antifungal activity and metabolism from fungi. The paddy field mycofloral diversity pattern was analyzed from the soil, and its physiochemical characteristics were also determined from the Kanyakumari district, Tamil Nadu, from 2016-2017. Totally twenty-seven fungal species were isolated from the paddy field soil. The physicochemical parameters of temperature, moisture content, pH, organic carbon, organic matter, and organic nitrogen were analyzed with significantly correlated. The most dominant fungal species include *Aspergillus flavipes, A.flavus, A.fumigatus, A.nidulans, A.niger, A.ochraceous, Curvularia lunata, Fusarium solani, Saccharomyces cerevisiae,* and *Trichoderma viride* were identified. Natural Products (NPs) synergistic effect was exploited to discover pairwise combinations with potential antifungal activity. A high-throughput screening approach with yeast revealed that NPs in molecules are the most promising novel synergies, namely, EUG+BER. This combination synergistically inhibits fungi, including human and crop pathogens such as *Candida albicans*, *Aspergillus fumigatus*, *Zymoseptoria tritici*, and *Botrytis cinerea*. This study was about screening pairwise NPs interactions as a tool to find novel antifungal synergies with the potential. Also, improved specificity may help manage fungal pathogens.

Keywords: Paddy field soil, physicochemical, fungal diversity, natural medicine, antifungal activity

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1. INTRODUCTION

The diversity of microorganisms that can survive environmental changes by synthesizing natural compounds to cope with harsh environments can be abundant in soil. Unique metabolites produced by microbes from the hard soil, such as in deserts with extraordinary environmental circumstances, could be used to make therapeutic goods, although some are harmful[1]. Fungi were estimated to produce 42% of all natural products produced by microorganisms[2]. Therefore, since their metabolites may provide a substitute for health security, fungi might be regarded as one of the main components of microbial production enterprises. Low molecular weight substances known as metabolites have a variety of biological functions that could be advantageous[3,4.] Fungi are fascinating and significant types of microorganisms that exist all over the planet and can be either saprophytes or parasites, depending on the environment. The potential antibiotic compounds to be secreted by soil mycoflora exists. The capacity of the fungi to develop quickly on organic substrates in arid, acidic, coarse-textured soils allowed them to access nutrients crucial for the biological control of plant pathogens. To understand the population dynamics of soil, mycoflora in the paddy field were analyzed. The population of fungi from the soil was identified with physicochemical parameters. When Alexander Fleming found that a substance made by a contaminated mould killed the bacterium in a petri dish seeded with *Staphylococcus aureus* in 1928, the era of microbiological drugs developed began. *Penicillium notatum* active ingredient was created. Other antibiotics, including streptomycin and chloramphenicol, were extracted from various bacterial and fungal fermentations using the same method. The fermentation process, which was first used to make beer and wine about 8000 years ago in ancient Egypt and Mesopotamia, can manufacture antibiotics. Similarly, *Penicillium roqueforti* has been used to make cheese for approximately 4000 years. Soy sauce making in Asia and bread baking are two other traditional fermentations[5,6.] Microbial primary or secondary metabolism can produce natural products (NPs) with great commercial value. The number of natural chemicals found has surpassed one million thanks to technical advancements in screening programs and techniques for separation and isolation[7]. Alkaloids, flavonoids, terpenoids, steroids, sugars, etc., are among them; 50–60% are created by plants, and 5% of these plant products have a microbial origin[8]. About 20–25% of the reported natural products showed biological activity; approximately 10% have been obtained from microbes. Numerous substances produced by microorganisms have a biological function. Fungi[9] generates about 9000 of the 22,500 bioactive chemicals obtained thus far from microbes. Consequently, fungi have played a major role in developing antibiotics and other medications to treat non-infectious diseases[10]. Antibiotics, pigments, growth hormones, anticancer drugs, and other microbial secondary metabolites are not necessary for the growth and development of microorganisms. Still, they have demonstrated a huge promise for improving human and animal health[11]. Bacteria, particularly actinobacteria, and fungi, produce a wide variety of small bioactive molecules with substantial promise for use in medicine among the microorganisms producing the mentioned chemicals[12]. The expression of these clusters would help exploit the chemical variety of microorganisms because these bioactive compounds are mostly created by activating inactive cryptic gene clusters under normal circumstances[13,14.] Antimicrobial medicines can be found in great abundance in natural products (NPs). They make up more than two-thirds of antibiotics used in clinical settings and half of anticancer medications[15]. Numerous secondary metabolites produced by plant endophytic and pathogenic fungi are crucial for virulence and competitiveness with other microorganisms. Due to their broad-spectrum action, some NPs can display strong biocidal activity against human pathogenic microorganisms. The discovery of marine fungi as a new source of fungal NPs in recent years has the potential to revolutionize the drug development process; however, marine fungi are currently a relatively untapped source of a variety of NPs. When germs, including bacteria, fungi, viruses, and parasites, develop resistance to one or more medications, it is called antimicrobial resistance. Following the use of several antimicrobial medicines in clinical settings, it has been noted that drug resistance is the main barrier to the effective treatment of infectious diseases. AMR's impact on humanity is difficult to measure, although drug-resistant bacterial infections alone are responsible for 25,000 deaths annually in the European Union[16]. Effective drug recovery responses require an effective drug delivery mechanism, a crucial component. Recently, it has been thought of using nanotechnology to create carriers for certain compounds. Drug bioavailability and therapeutic potential are greatly increased using nanocarriers and innovative formulations that enrich the target location[17.] However, the overview of these substances and the monotony of their modes of action have resulted in developing resistance to these substances. As a result, controlling pathogenic bacteria has gotten harder over the past few decades, exacerbating food production instability and insecurity. Drug discovery is constantly needed due to drug resistance. An attempt is being made to understand better the mechanisms underpinning host-microbe interactions, pathogen population dynamics, and medication modes of action to manage the risk of developing antimicrobial compound resistance. Here, several natural compounds generated from fungi are described in terms of their structures, biosynthesis, and antibacterial properties. This effort to search for natural products is known as bioprospecting. The discipline of pharmacognosy, the study of natural products with biological activity, provides the tools to identify, select and process natural products destined for medicinal use. Usually, a natural extract has some form of biological activity that can be detected and attributed to a single compound or a set of related compounds produced by the organism. These active compounds can be used in drug discovery and development directly as they are, or they may be synthetically modified to enhance biological properties or reduce side effects. The present study aimed to find the Diversity of mycoflora and their bioactive compounds in various diseases for healing human ailments and plant pathogens.

2. MATERIALS AND METHODS

2.1. Sample Collection

They are collecting soil samples of paddy fields in Kanyakumari district, Tamil Nadu, India. It is situated at 8.3235°N, 77.3324°E, the southernmost point. Tropical and monsoonal weather is typical of the region. In the years 2016 - 2017, there was a fair amount of rain. The soil samples were randomly collected at a depth of 15 cm from various locations within the paddy field.

2.2. Soil Physicochemical analysis

2.2.1. Temperature (°C)[18]

The soil temperature was analyzed with the help of a thermometer, then read the values and recorded; the temperature was expressed as oC.

2.2.2. Moisture[19]

$$\text{Calculation: \% of Moisture} = \frac{\text{Air dried} - \text{Oven dried}}{\text{Oven dried}} \times 100$$

2.2.3. pH[20]

The digital pH meter was calibrated using a standard buffer solution at the pH value of 4.01 and 7.00. The pH readings were taken by immersing the glass electrode/probe into the solution and recording the reading.

2.2.4. Organic carbon[21]

The samples were oven-dried at 105 ◦C for 24 h until the constant mass was acquired. Then, dried the samples were cooled in a desiccator, and the final mass was measured.

One gram of soil samples was treated with 5 mL of concentrated H2SO4 for four h, then with 5 mL of 0.5 M K2Cr2O7. The mixture was heated at 150-160◦C for 5 min and then cooled at room temperature. Next, the solution was moved into a conical flask with 100 mL of deionized water. The unreacted K2Cr2O7 was determined by titrating with 0.25 M FeSO4. The endpoint approached when the solution changed from dark green to blue to reddish-brown.

$$\text{Calculation: \% of Organic carbon} = M \times \frac{V_1 - V_2}{\text{Mass of soil}} \times 0.39.$$

Where,
M=Concentration of FeSO4;
V=Volume of blank;
V2=Volume of FeSO4
0.39=Constant

2.2.5. Organic Matter[22]

One kg of soil samples was taken in a 250ml conical flask with 10 ml of 1N K2Cr2O7 and swirling gently. Then 20 ml of concentrated sulphuric acid was added, and suddenly the flask swirled and placed on a hot plate at 135oC. After the flask was cooled and diluted to deionized water titrated against 0.4N ferric sulfate as an indicator at the endpoint, the greenish color changed to dark green. Finally, the ferrous sulfate was added to dropwise blue-green to change in reddish-grey and was recorded at the endpoint as maintain blank to standardize the FeSO4 solution.

Calculations

The percentage of carbon was determined from the following formula:

$$\text{Organic matter (\%)} = 100 \times \frac{3\left(1 - \frac{T}{S}\right)}{W}$$

Where:
S = Volume of FeSO4 used in blank titration (ml)
W = Weight of soil in grams,
T = ml of FASS = Ferrous solution with blank titration (ml)

2.2.6. Organic Nitrogen[23]

One kg of soil samples was mixed in hundred ml distilled water in a test tube. In addition, 1.5 ml of NaOH and 1 ml of Nessler's reagent were added, and readings were at 540 nm using a spectrophotometer. The results were expressed in mg/ml.Test OD/test OD conc. Of Std. 100/ volume of a sample taken.

2.3. Isolation soil of fungi[24]

The collected soil sample (Soil dilution method) is diluted with 1g of soil in 10 ml of sterile distilled water. 1ml of suspension was added to sterile Petri plates in triplicates containing sterile Potato Dextrose Agar plates were incubated at 28°C for 2-5 days.

2.4. Identification of fungi[25-28]

The isolated fungi were identified to the genus and the species level based on their morphological characteristics. Then, microscopic analysis was analyzed using slide cultures, the most taxonomic guides, and standard procedures.

2.5. Different strains and their improvement[29]

Saccharomyces cerevisiae was derived from sub-culturing three times on YPD agar supplemented with 40 µg/mL−1 ethidium

bromide. For experimental purposes, the yeasts were streaked onto YPD agar maintained at 37°C glycerol stocks and cultured for at least 48h before single colonies were picked for sub-culture to the broth.

2.6. NPs production from fungi

2.6.2. Extraction of Berberine[30,31]

Berberine, a quaternary protoberberine alkaloid (QPA), is the most common alkaloid in its class. According to current research, the QPA alkaloids can be isolated from their matrix using various approaches. The interconversion reaction between the protoberberine salt and the base provides the basis for these approaches. The salts are water soluble, stable in acidic and neutral conditions, and soluble in organic solvents. Thus, the protoberberine salts are transformed in their respective bases throughout the extraction phase and then extracted in organic solvents. Berberine extraction procedures such as maceration, percolation, Soxhlet, and cold or hot continuous extraction use various solvent systems such as methanol, ethanol, chloroform, aqueous, and acidified solutions. Berberine's sensitivity to light and heat makes extraction difficult.

2.7. Extraction of Eugenol[32]

Xue-Song Company (Jiangxi, China) provided natural eugenol (99%purity) isolated from clove buds, dissolved in ethanol. The stock solution was kept at four °C until it was used. At 4°C, the culture was kept in the dark on PDA media (200 g boiling potato, 20 g dextrose, 20 g agar, 1 L). Conidia from a 7-day-old PDA culture were counted using a hemocytometer after being rinsed with 0.01% Tween-20 solution. A suspension containing 5 107 conidia/mL was made. The conidia were inoculated into YES broth (20 g yeast extract, 150 g sucrose, 0.5 g MgSO4.7H2O, 1L) at a final concentration of 106 conidia/mL. After diluting the eugenol stock solution with ethanol to a concentration of 80 mM, 500 L of the diluted eugenol stock was added to 50 mL of YES broth, yielding a final eugenol concentration of 0.80 mM. The control cultures were given the same treatment but were not given eugenol. Each culture was incubated for five days at 28°C in the dark. After that, the mycelia were harvested. Each therapy was carried out three times.

2.8. Antifungal activity[33]

Agar well–diffusion method was followed for the determination of antifungal activity. Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 48 hours old of Aspergillus fumigatus, Botrytis cinerea, Candida albicans, and Zymoseptoria tritici respective fungi were procured from Indian Biotrack Research Institute, Thanjavur, Tamil Nadu, India. Agar wells (5mm diameter) were made in each plate using a sterile cork borer. About standard, 25, 50, 75, and 100µl of Berberine, Eugenol, and its synergies extracts were added using sterilized dropping pipettes into the wells. Plates were left for 1 hour to allow a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. The plates were incubated upright at 28˚C ± 2˚C for fungi. Results were recorded as the presence or absence of an inhibition zone. Triplicates were maintained, and the average values were recorded for antifungal activity.

2.9. High-Throughput Screening[33]

For high-throughput screens, the test compounds of 750µM eugenol and 350 µM berberine were assayed in pairwise combinations against off, comprising chemicals at ten mM, dissolved in DMSO. For the screens, aliquots (1 µL) of each library compound were combined with 49 µL YPD and added to 96-well microtiter plates (Greiner Bio-One). Next, aliquots (50 µL) of yeast cell suspension (prepared as described above) containing one of the four test compounds were added to the 50 µL library-compound preparations in the microtiter plates. It gave final concentrations of 100 µM of each library compound in 100 µL total per well. Solvent-matched controls at 0.35% DMSO or 0.3% ethanol (70%) were used for control assays without added compounds. The growth with added compounds was expressed as a percentage of control growth and the compounds.

2.10. Statistical analysis

Excel 2010 was used for initially analyzed with data and creating a database for drawing related charts. Statistical analyses were performed using standard procedures for a randomized plot design (SPSS 22.0, SPSS Inc., Chicago, IL, USA). The statistical analysis of soil physicochemical properties with fungal populations was performed with SPSS.

3. RESULTS

3.1. Soil physicochemical parameters

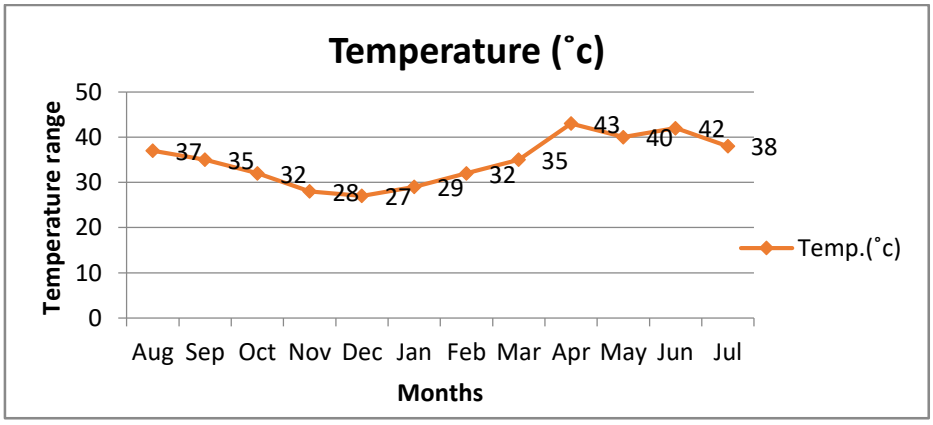


Fig 1: Month-wise analysis of temperature of the soil of paddy field

The paddy field soil's physicochemical properties, such as temperature, moisture, pH, organic carbon, organic matter, and organic nitrogen, were determined for every month of 2016-2017. In addition, the monthly variations of soil temperature were noted periodically, such as August (37°C), September (34°C) and March (35°C), October and February (32°C), November (28°C), December (27°C), January (29°C), April (43°C), May (40°C), June (42°C) and July (38°C) month temperature were recorded respectively (Figure-1).

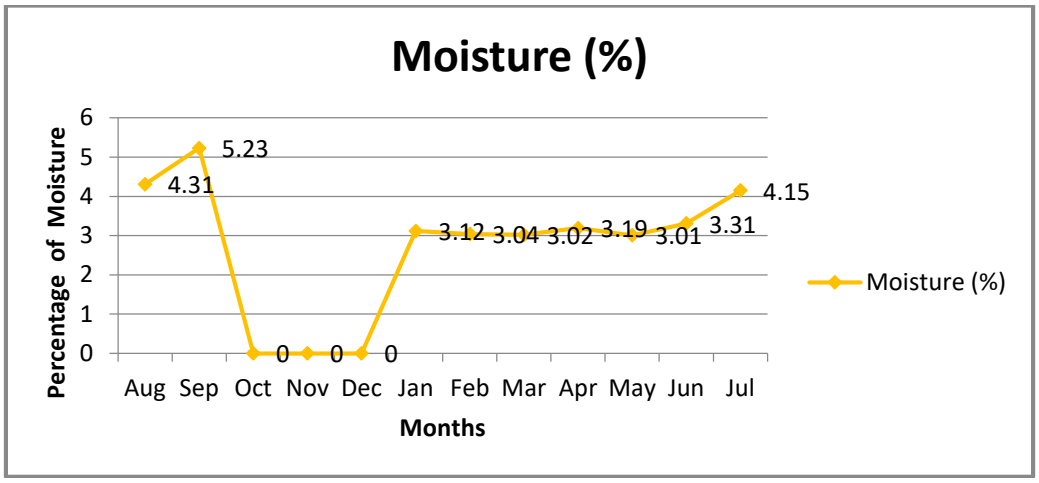


Fig 2: Month-wise analysis of moisture level of paddy field soil

The percentage of moisture was measured with the months such as August (40.31%), September (50.23%), January (30.12%), February (30.04%), March (30.02%), April (30.19%), May (30.01%), June (30.31%) and July (40.15%) but October, November and December months was fully saturated moisture content (Figure-2).

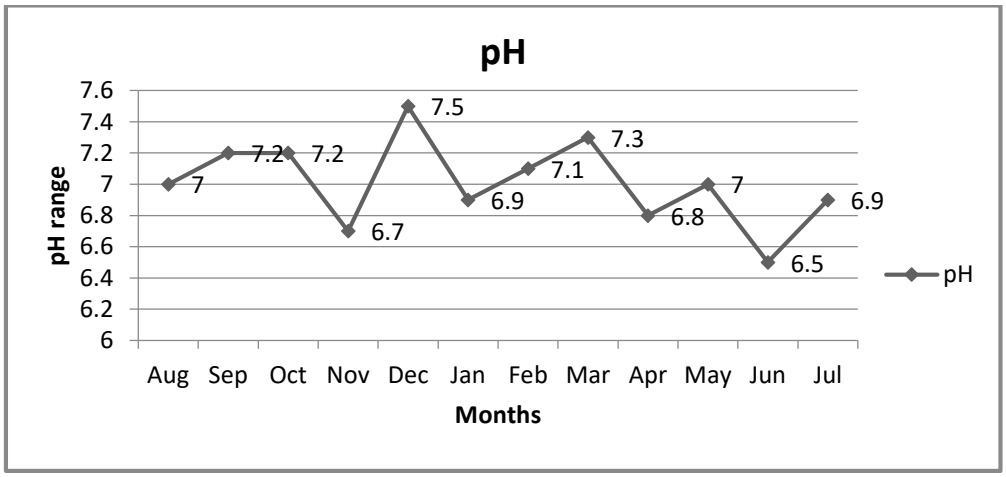


Fig 3: Month-wise analysis of pH of the soil of the paddy field

Each month's pH content was in August and May (7.0), September and October (7.2), November (6.7), December (7.5), January and July (6.9), February (7.1), March (7.3), April (6.8), May (7.0) and June (6.5) respectively (Figure-3).

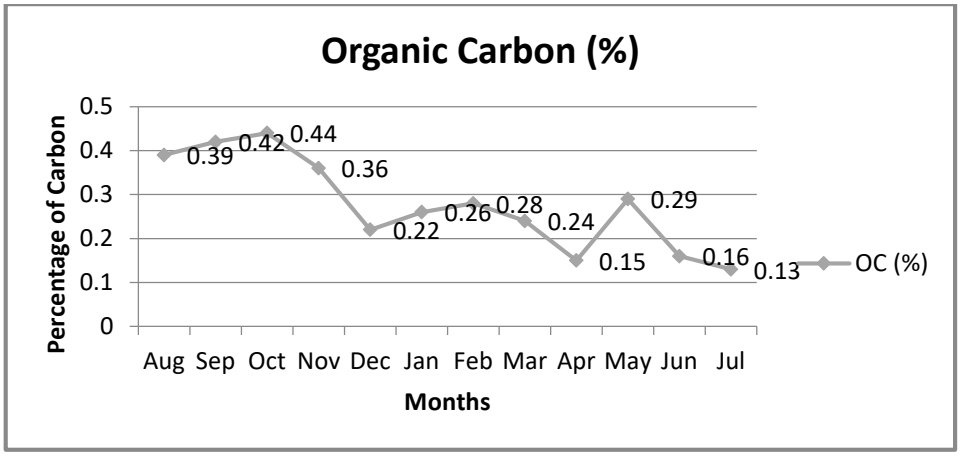


Fig 4: Month-wise organic carbon of paddy field soil

Organic carbon was estimated in percentage for each month, such as August (0.39%), September (0.42%), October (0.44%), November (0.36%), December (0.22%), January (0.26%), February (0.28%), March (0.24%), April (0.15%), May (0.29%), June (0.16%) and July (0.13%) month were observed (Figure-4).

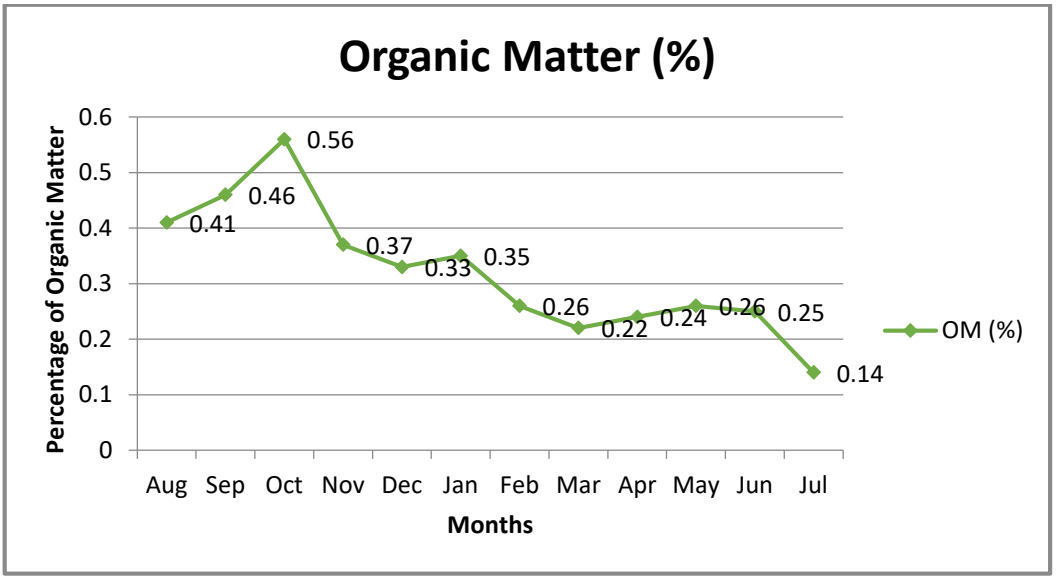


Fig 5: Month-wise analysis of the organic matter of paddy field soil

Organic matters were estimated in percentage for each month, such as August (0.41%), September (0.46%), October (0.56%), November (0.37%), December (0.33%), January (0.35%), February (0.26%), March (0.22%), April (0.24%), May (0.26%), June (0.25%) and July (0.14%) found to be recorded respectively (Figure-5).

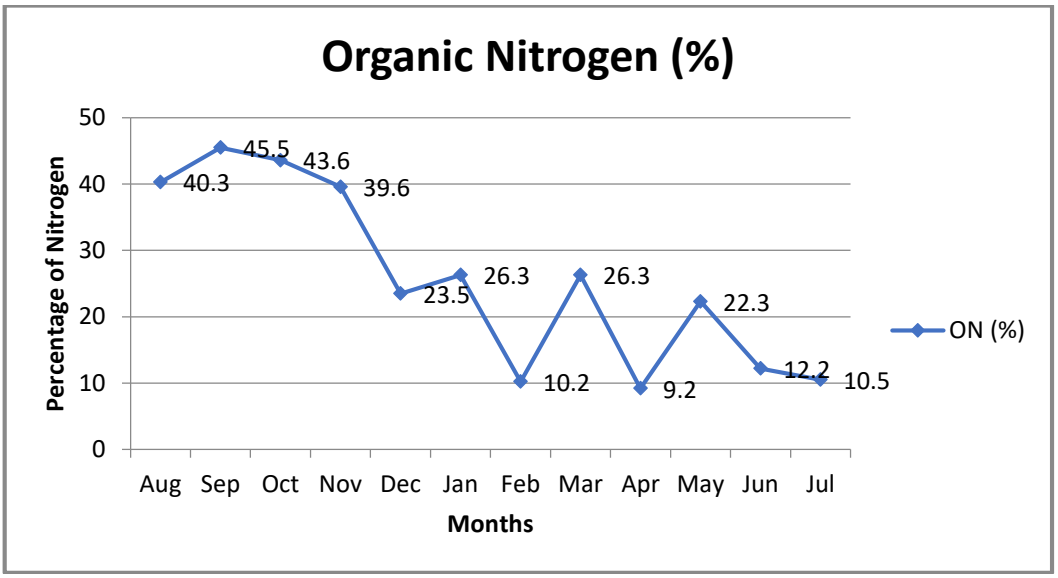


Fig 6: Analysis of organic nitrogen of paddy field soil

The percentage of organic nitrogen was found for each month, such as August (40.3%), September (45.5%), October (43.6%), November (39.6%), December (23.5%), January (26.3%), February (10.2%), March (26.3%), April (09.2%), May (22.3%), June (12.2%) and July (10.5%) month represented from the soil sample respectively (Figure-6).

3.2. Microbial diversity

3.2.1. Microbial load

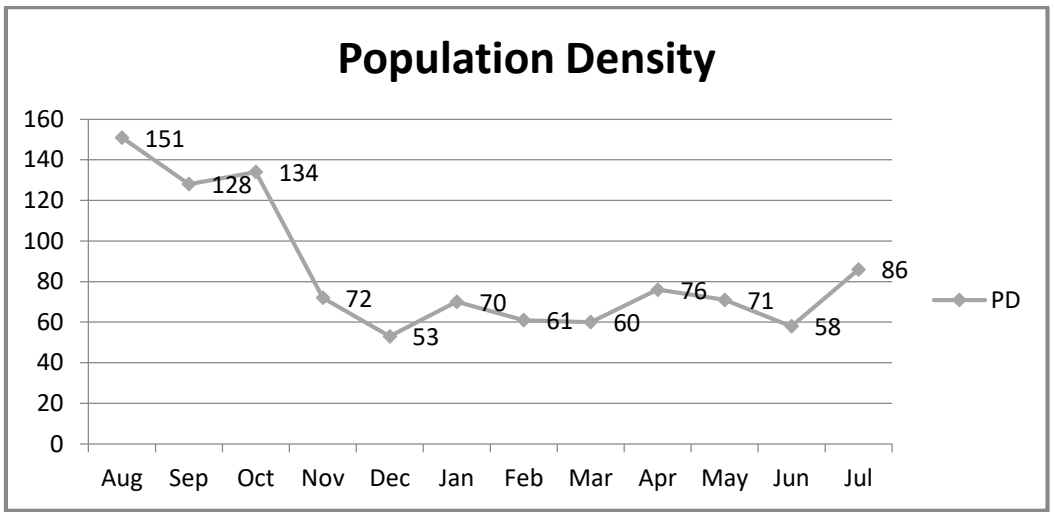
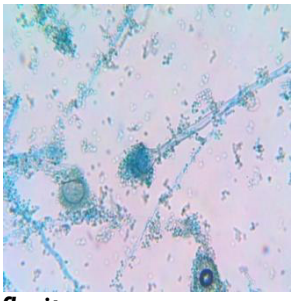


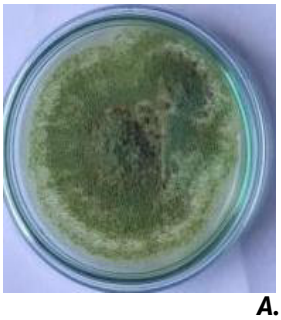
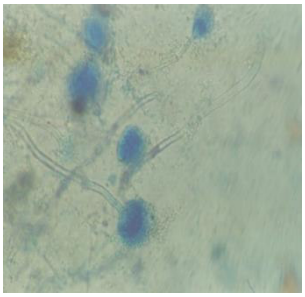
Fig 7: Analysis of population density of paddy field soil with different months

The population density of isolated fungal colonies was found for every month, such as August (166), September (137), October (152), November (78), December (62), January (77), February (66), March (67), April (85), May (75), June (73) and July (97) were analyzed respectively (Figure-7).

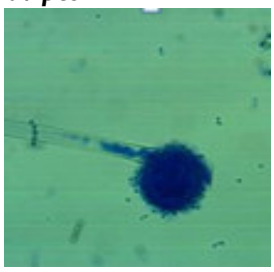
3.2.2. Characterization of fungi

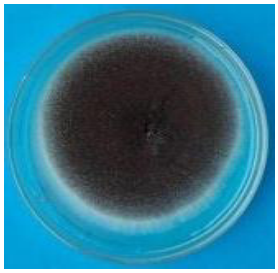
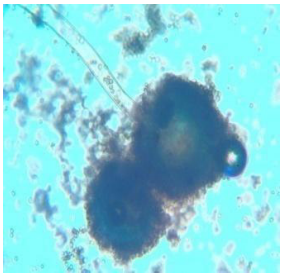
Aspergillus flavipes

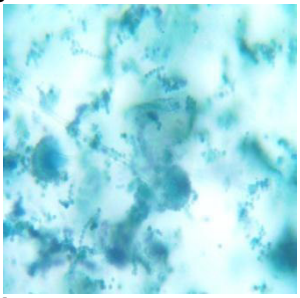
A. flavus

A. fumigatus

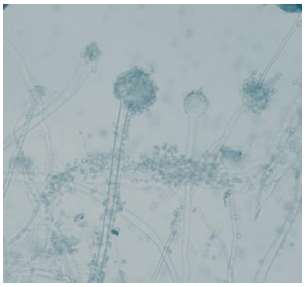



A. niger

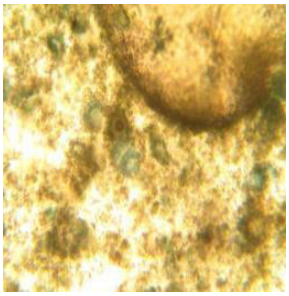



A. nidulans

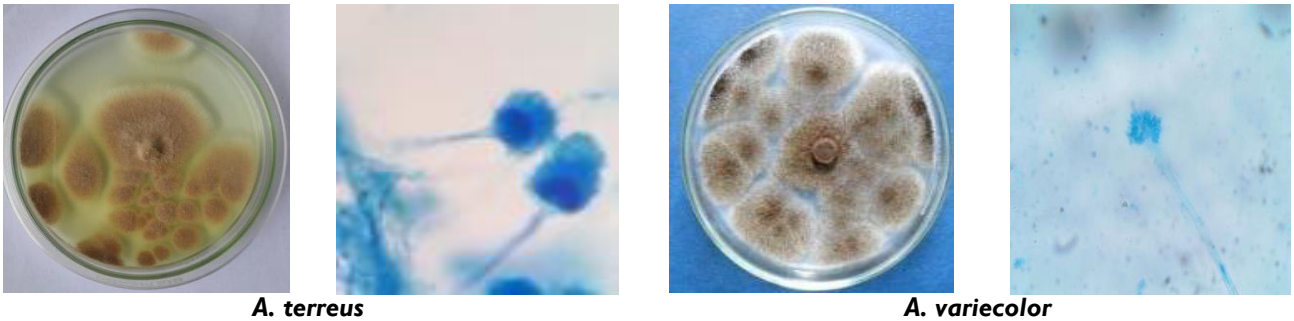

A. ochraceus

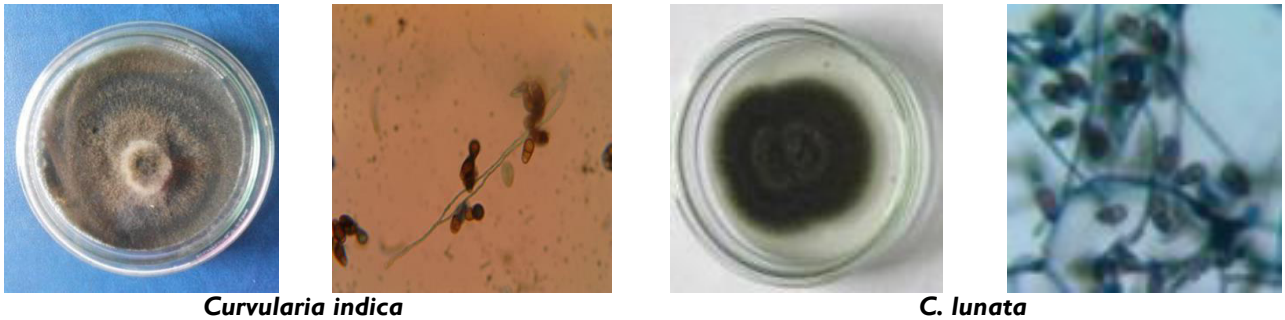
A. oryzae

A. spinulosus

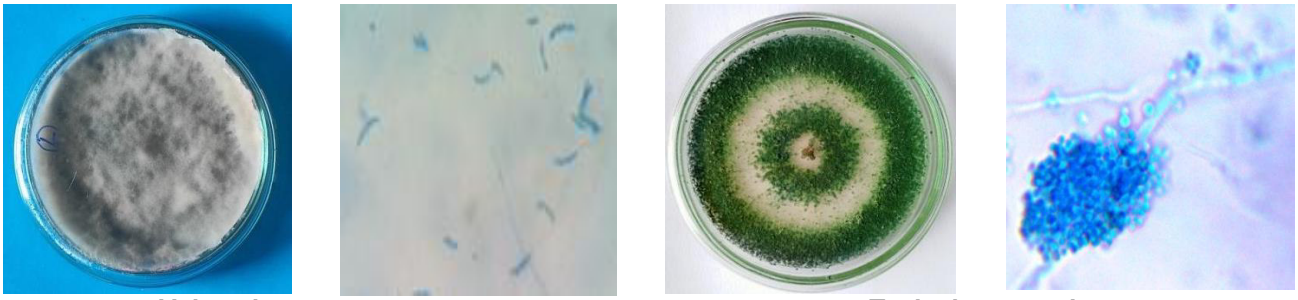


A. terreus *A. variecolor*

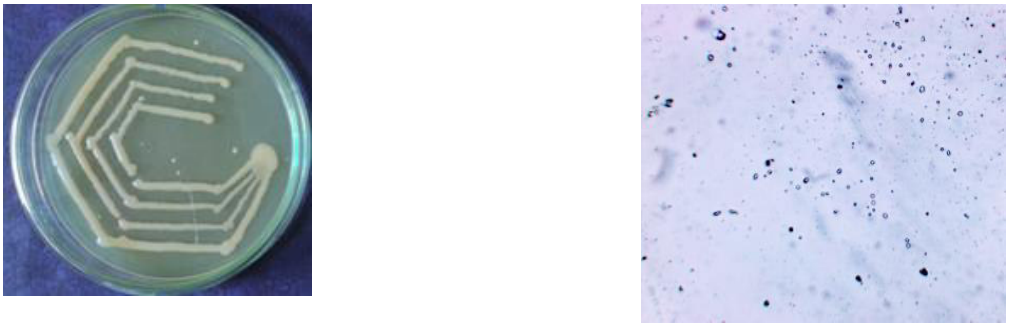


Curvularia indica *C. lunata*

Fusarium oxysporum *F. solani*



Helminthosporium oryzae *Trichoderma viride*



Saccharomyces cerevisiae

Fig 8: Identification of isolated fungi from paddy field soil of Kanyakumari

The isolated fungi from paddy field soil of the Kanyakumari district for the period of 2016-2017 found 27 fungal species such as *Aspergillus awamori, A. candidus, A.flavipes, A.fumigatus, A.flavus, A.nidulans, A.niger, A.ochraceous, A.oryzae, A.rugulosus, A.terreus, A.ustus, Curvulariaindica, C.lunata, Fusariumsolani, F.oxysporum, A.variecolor, Helminthosporiumvelutinum, Mucoralboarter, Penicilliumcitrinum, P. chrysogenum, P.funiculosum, Rhizopusnigricans, Saccharomyces cerevisiae, Trichodermaviride, T.harzianum, and Verticilliumsp*. were identified fungi and belonged to the family Trichomaceae, Pleosporaceae, Nectriaceae, Mucoraceae, Hypocreaceae, *Saccharomycetaceae* and Plectosphaerellaceae (Figure-8).

3.2.3. Statistical Analysis

Table 1: Pearson correlation matrix between fungal population and physico-chemical characteristic of paddy field soil

Physicochemical parameters	Temperature (˚C)	Moisture (%)	pH	OC (%)	OM (%)	ON (%)	PD
Temperature (˚C)	1						
Moisture (%)	*0.599504	1					
pH	-0.46725	-0.19452	1				
OC (%)	-0.40021	-0.15975	0.304053	1			
OM (%)	-0.4261	-0.31537	0.272254	0.883149	1		
ON (%)	*0.43276	-0.19077	0.299933	0.907615	0.862234	1	
PD	0.084212	0.244787	0.130077	0.706435	0.689639	0.692031	1

FS = fully saturated OC = organic carbon; OM = organic matter; ON = organic nitrogen; PD = population density; p>0.005 the percentage level of significant

3.2.4. Monthly variation of fungi

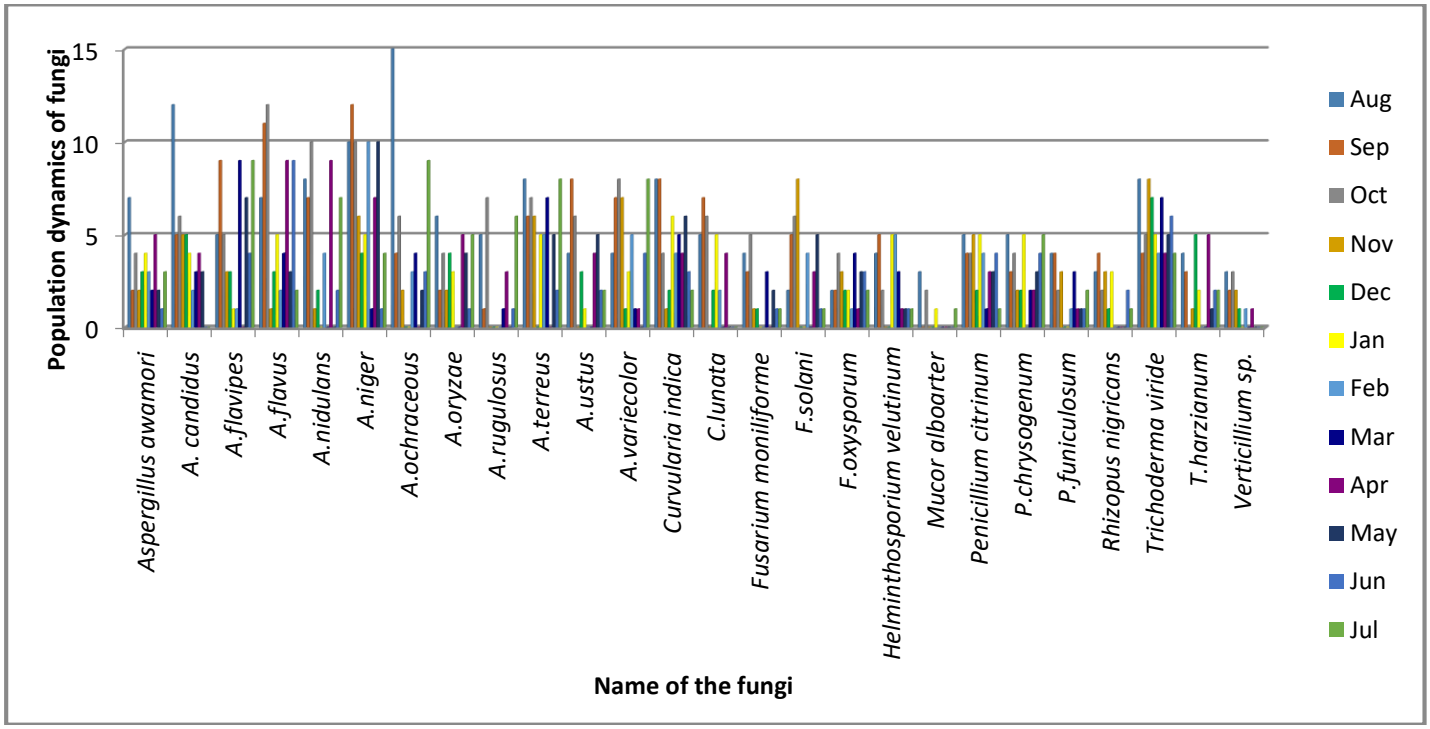


Fig 9: Monthly variation in the population of soil fungi (No. of colonies×103 g-1 dry wt of the soil)

The monthly variations in the population of soil fungi were correlated with the physicochemical parameters; the other months of temperature, moisture, and pH are significantly represented, respectively (Table-1 & Figure - 9).

3.3. Combination of Natural medicine

The two selected combinations' wider efficiencies were tested against fungal pathogens: *Aspergillus fumigatus, Botrytis cinerea, Candida albicans, C. glabrata and Z. tritici.* In addition, the EUG + BER combination retained synergistic activity against *C. albicans* showing a significantly greater observed. Therefore, the combined effect would be expected from an additive interaction of the observed individual-NP effects.

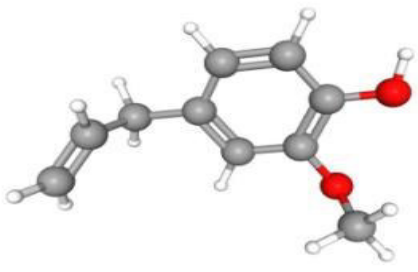


Fig 10: Structure of Berberine

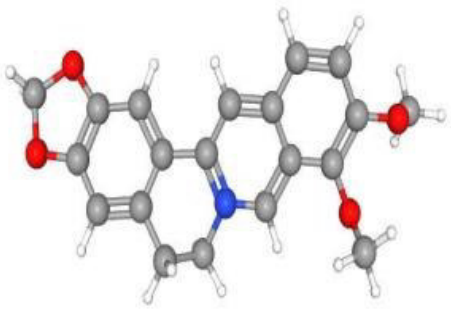


Fig 11: Structure of Eugenol

3.4. Antifungal activity of Natural compounds

The antifungal activity of Berberine and Eugenol against the clinical pathogens are *Aspergillus fumigatus, Botrytis cinerea, Candida albicans, and Zymoseptoria tritici.* The combined effect of different concentrations of 25, 50, 75, and 100µl of Berberine and Eugenol was treated against pathogens and produced following zones of inhibition such as *Aspergillus fumigatus* (0.50±0.20, 0.90±0.05, 0.88±0.30 and 1.14±0.04mm), *Botrytis cinerea* (0.91±0.30, 0.95±0.01, 0.93±0.02 and 1.04±0.06mm*), Candida albicans*(1.13±0.20, 1.45±0.02, 1.62±0.03 and 1.90±0.80mm) and *Zymoseptoria tritici* (0.95±0.40, 1.10±0.40, 1.19±0.02 and 1.53±0.06mm) were recorded respectively (Figure – 12).

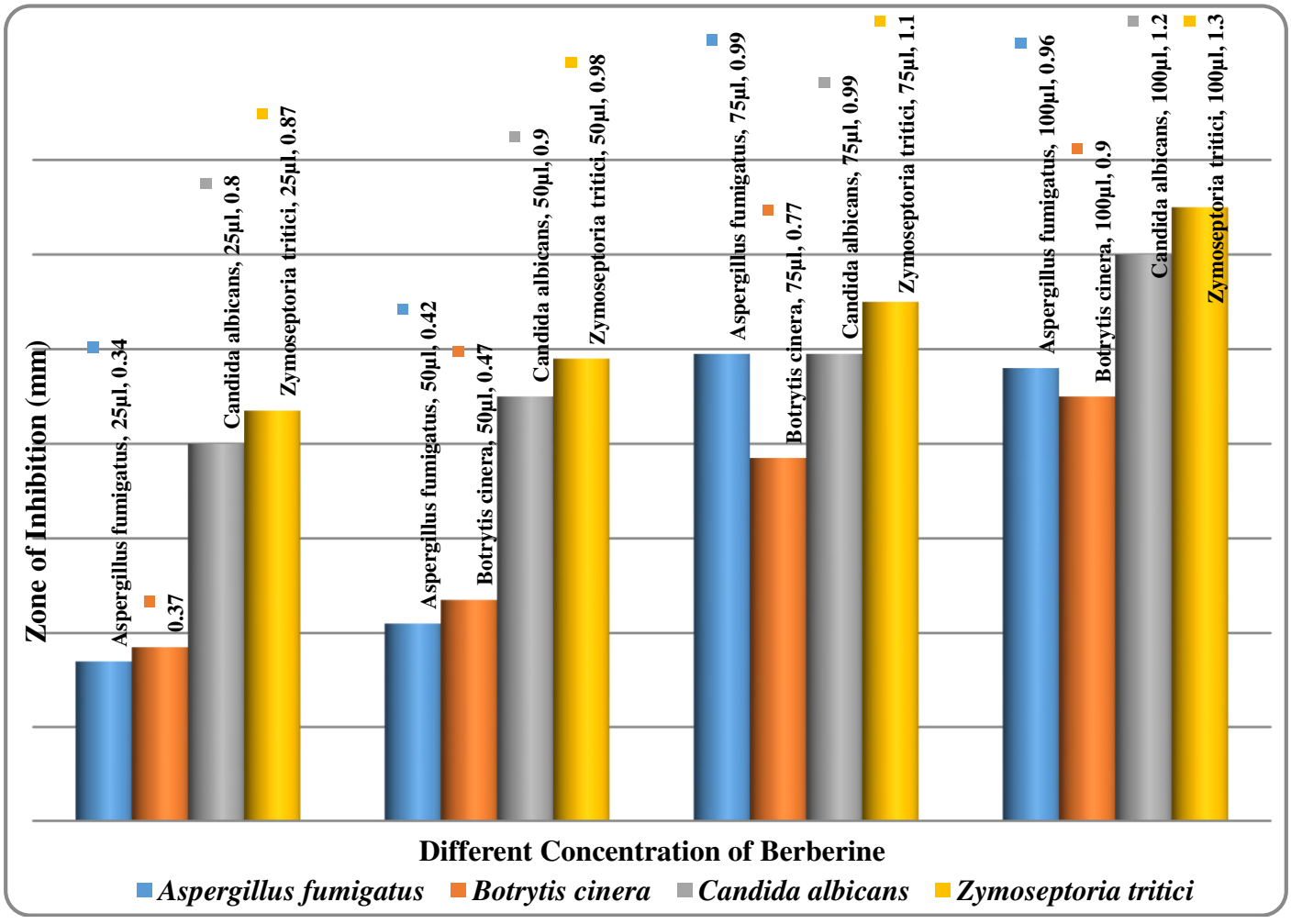


Fig12: Antifungal activity of Berberine from fungi against clinical fungal pathogen

The different concentration of 25, 50, 75, and 100µl of eugenol was treated against clinical pathogens and produced following zones of inhibition such as *Aspergillus fumigatus* (0.34±0.10, 0.42±0.32, 0.99±0.34 and 0.96±0.78mm), *Botrytis cinerea* (0.37±0.67, 0.47±0.56, 0.77±0.20 and 0.90±0.02mm), *Candida albicans* (0.80±0.10, 0.90±0.30, 0.99±0.32 and 1.20±0.20mm) and *Zymoseptoria tritici* (0.87±0.32, 0.98±0.30, 1.10±0.15 and 1.30±0.20mm) were recorded respectively (Figure – 13).

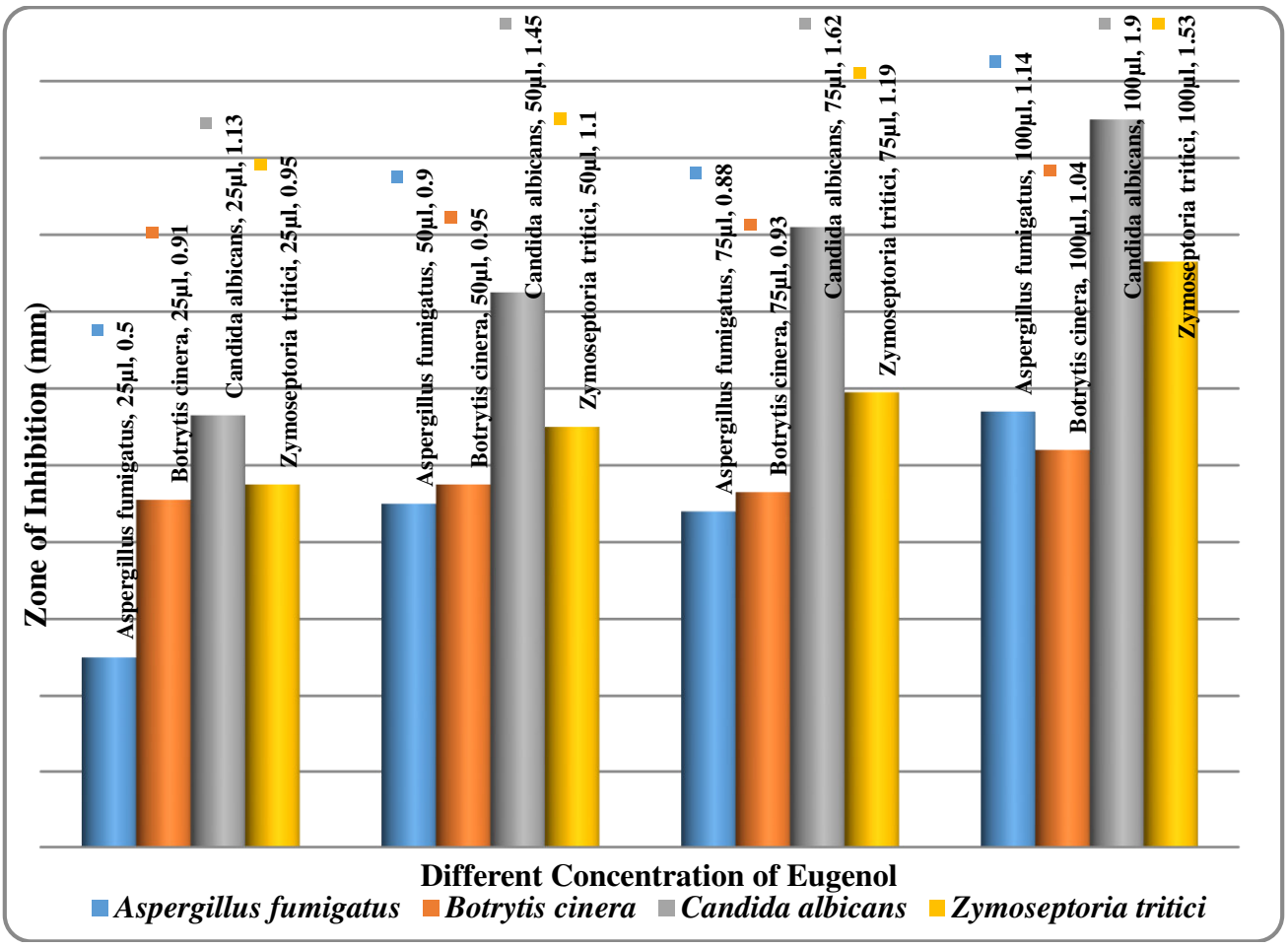


Fig 13: Antifungal activity of Eugenol from fungi against clinical fungal pathogen

The different concentration of 25, 50, 75, and 100µl synergies of Berberine and eugenol was treated against clinical pathogens and produced following zones of inhibition such as *Aspergillus fumigatus* (1.0±0.20, 2.2±0.5, 3.8±0.30 and 5.4±0.04mm), *Botrytis cinerea* (4.0±0.30, 6.5±0.01, 7.3±0.02 and 8.4±0.06mm), *Candida albicans* (9.0±0.20, 11.5±0.02, 12.2±0.03 and 13.0±0.80mm) and *Zymoseptoria tritici* (10.5±0.40, 12.0±0.40, 13.5±0.02 and 14.3±0.06mm) were recorded respectively (Figure – 14).

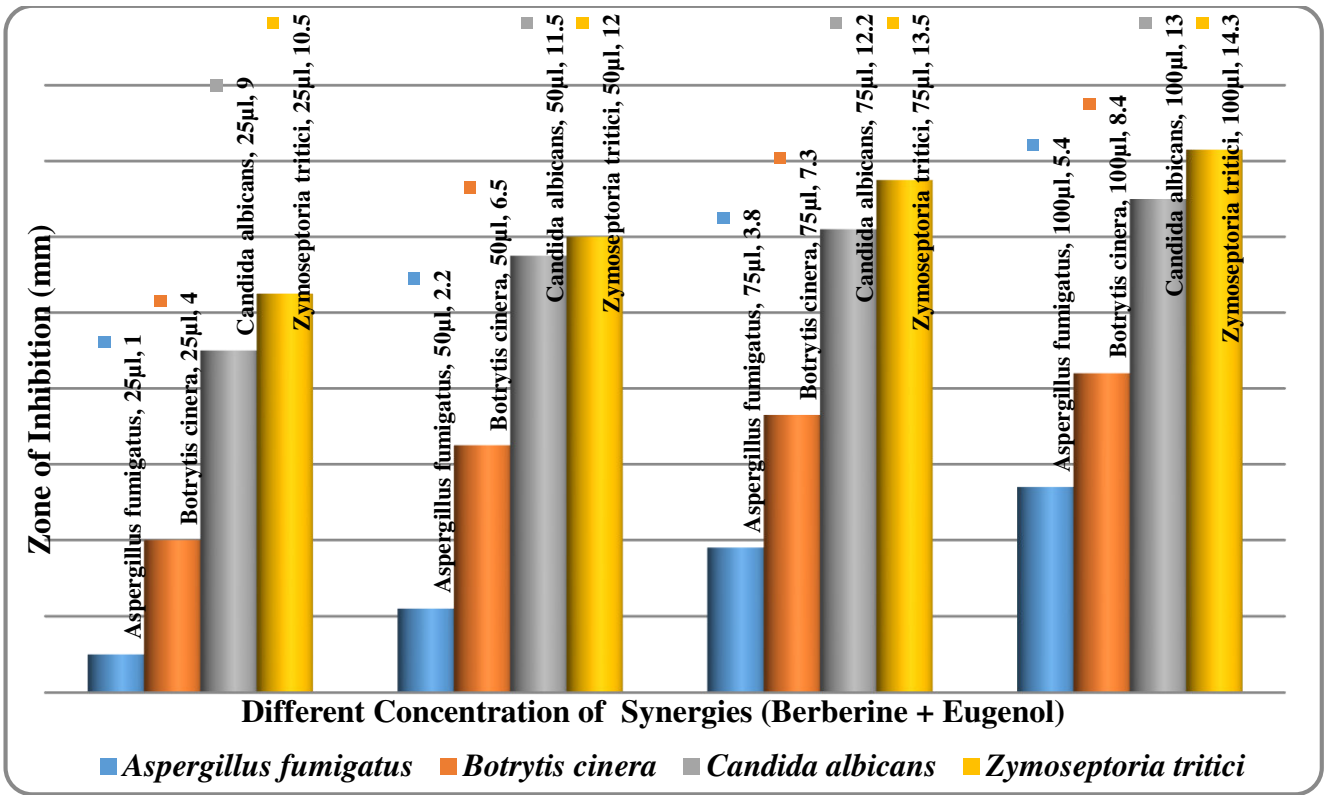


Fig 14: Antifungal activity of synergistic (Berberine+Eugenol) against clinical fungal pathogen

4. DISCUSSION

The important factors influencing the variation in the population of fungi in the present study could be temperature, organic nitrogen, and soil moisture content. The paddy field soil was subjected to disturbances such as irrigation, fertilization, and agricultural practices resulting in more homogeneity of soil which did not allow relatively wide fluctuation in the fungi population.The chemical composition of the soil and the diversity of the soil microorganisms can be impacted by agricultural management practises[34,35]. Several physico-chemical characteristics of agricultural soils have an impact on the mycoflora there. Significant effects on a soil's fungal variety are caused by its organic content, moisture content, and pH[36].Soil fungi are related to plant roots, providing important nutrients like nitrogen, phosphorus, and potassium. Fungi can colonize the upper parts of plants and provide many benefits, including decomposing from dead material drought tolerance, heat tolerance, and resistance to insects and plant diseases. However, fungi help plants to absorb nutrients and water more efficiently. Fungi are, therefore, a vital part of the huge agricultural industry. It has been reported that the density of the fungal population occurred during the rainy season when the soil moisture was significantly high[37]. A similar study was done in different parts of India where *Aspergillus sp. and Penicillium sp*. were dominant fungal genera[38] in Uttar Pradesh. Our results were supportedby early studies [39,40]. The fungi were isolated from paddy fields at Suryamaninagar, Agartala, Tripura[41]. The isolated fungi were identified based on cultural, microscopic, and morphological characteristics. It is known that PDA is the universal medium most widely used in the isolation of fungi, having a complete nutritional basis[42]. This is probably why colony development was faster compared to other media. Earlier work supported that the maximum growth of fungi was recorded in a potato dextrose agar medium[43].Microbial diversity of paddy cultivated soil samples have been identified as fungal colonies such as *Aspergillus niger, A. flavus, A.fumigatus, A. terreus, A. carbonarius, A. nidulans, Alternaria sp., Penicillium sp. and Talaromyces sp*. and the genera of *Aspergillus, Alternaria, Penicillium, Talaromyces* and unknown species of Penicillium and *Saccharomyces cerevisiae* are a maximum number of colonies, and similar studies were reported[44].The colony morphologies of soil fungi are significantly influenced by the growing medium used; *Alternaria, Aspergillus, Penicillium, and Talaromyces* genera have all been effectively cultivated on RB media[45].The dominance and prevalence of the species have been reported from India and in various other parts of the world[46]. The species of Aspergillus have been reported to have the most tolerance to adverse conditions in the laboratory[47], and species of *Aspergillus and Penicillium* were tolerant to a wide range of environmental conditions[48]. Eighteen species belong to 4 genera from Kadegaon Tehsil, Sangli District, Maharastra, India. The *Aspergillus* genera were dominant[49,50]. Similar results were reported in the present investigation. Ten fungal species representing seven genera were found in the rhizospheric soils of paddy, pulse, ragi, sugarcane, vegetable, and banana farms in the Nanjangud taluk of Mysore district, Karnataka. *Aspergillus, Alternaria, Mucor, Curvularia, Fusarium, Penicillium, and Rhizopus* have all been reported. 27 species from 19 different genera have been isolated, with *Aspergillus, Fusarium, and Alternaria* being the most prevalent[51]. The fungal community of CK soil diverged from those of rhizosphere soils in abundances of both Ascomycota and unidentified phylum; the former dominated in rhizosphere soil, especially in TCC soil (70.0%), and the latter dominated in CK soil (39.6%). The abundance of Basidiomycota was highest in the FCC community (18.5%)[52]. Eugenol is already used in pharmaceutical products, as a food preservative, in agriculture, and cosmetics and can be administered at 2.5 mg/kg body weight per day in humans[53]. Similarly, numerous studies have supported the application of Berberine for therapeutic purposes in humans concerning different conditions, owing partly to good absorption and toxicity properties[54,55]. A variety of significant pathogenic and spoilage ascomycete fungi were suppressed synergistically, including efficacy against *C. albicans* biofilms (EUG + BER). Additionally, synergy was still seen in isolates of *C. albicans and A. fumigatus* resistant to azoles. It suggests possible significance for the clinical context where azoles are important medications for treating invasive infections[56]. Recently, 71 compounds out of 142 examined in a study of natural products with anti-*Candida albicans* activity met the criterion for having antifungal activity by having minimum inhibitory concentrations (MIC) values below 8 mg/mL[57].Gaining inhibitory activity through synergy may be crucial for overcoming the problem of promiscuous behaviors linked to several examined NPs[58]. Since synergy often focuses on a shared target of the different medicines, this increase in activity adds specificity. In order to protect possible translational uses, anti-action promiscuity in regular NP-screening[59].Amphotericin B, a traditional polyene antifungal product of *Streptomyces nodosus*, is also used to treat life-threatening fungal infections from *Aspergillus* species. It works particularly well in patients who have had organ transplants, received intensive chemotherapy, or have acquired immunodeficiency syndrome[60-62].In China, Berberine has been used for thousands of years to treat diarrhea, and it is still frequently utilized in modern medicine. The effects of Berberine on fungi have been the subject of numerous investigations. The Candida species, including *C. albicans, C. krusei, C. glabrata, and C. dubliniensis,* are said to be inhibited by Berberine, with MICs ranging from 10 to 160 g/mL[63,64].Berberine also works well with other antifungal medications like amphotericin B and terbinafine. In a mouse model, the combination of Berberine and amphotericin B lowered the amphotericin B dose by around 75%[65], and 100 g/mL berberine can effectively supplement terbinafine's antifungal activity[66]. Clove Oleoresin combined with concentrated sugar has a powerful fungicidal action on *C.albicans, P.citrinum, A.niger, and T.mentagrophytes*[67]. Mycelial inhibition was concentration-dependent, as colonies' diameters shrank as the amount of EO increased. Except for *Mucor and Aspergillus*, all other fungal strains were inhibited significantly at 1ml concentration [68.] The present investigation suggested that Berberine and eugenol's combined effect was better antifungal activity than the individual compounds. Obliviously, the synergistic effect of fungal compounds which responsible for various diseases. The fungal bioactive compounds found to be the specific targeted natural compounds were enormous active principles in various diseases and disorders of human life.

5. THE FUTURE OF MYCOFLORAL ECOLOGY

Mycofloral significance plays a major role in conserving ecology and the environment. We can challenge the mycoflora to alter the environment. That could be harnessed to engineer our planet and health. However, the current knowledge of the metabolic mechanism and evolutionary process in most microbial ecosystem dynamics is extremely limited. The enormous population sizes and rapid growth rates of microorganisms mean that microbial ecology may transform the fields of ecology and evolution to test specific hypotheses

the specific compounds for the role of variations context molecules in future generations.

6. CONCLUSION

It can be concluded that fungi are very successful inhabitants of the soil. The integrated approach to soil health assumes that soil is living system and health results from the interaction between different processes and properties with a strong effect on the activity of soil microbiota. The soil physicochemical properties were indispensable for the population of microflora and enhancing soil conservation and human fertility. As per the statistics of the research work, this promoted the ecosystem in the sustainable growth of the fungal community and the targeted count measured for the pathogens significantly. The fungal synergistic compounds were responsible for particular pathogens in particular diseases.

7. AUTHORS CONTRIBUTION STATEMENT

Dr. S. Murugesan designed and finalized the manuscript of the study, Dr. A. Panneerselvam and Dr. V. Ambikapathy provided valuable suggestions for this work, Mrs. S. Rajathi collected the sample and analyzed the work and prepared the draft manuscript, Mr. P. Prakash helped for analysis and alignment of manuscript, Dr. Shijila Rani, A.S. and Dr. S. Babu discussed the methodology. Finally, all authors read and approved the final version of the manuscript.

8. ACKNOWLEDGEMENTS

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Jadon KS, Singh SK. and Rakesh P. New and Future Developments in Microbial Biotechnology and Bioengineering. Recent Advances in Application of Fungi and Fungal Metabolites: Environmental and Industrial Aspects, Potentials of metabolites of soil fungi. 2020: 1-9.
2. Demain AL. Importance of natural microbial products and the need to revitalize their discovery. J IndMicrobiolBiotechnol. 2014;41(2):185-201. doi: 10.1007/s10295-013-1325-z, PMID 23990168.
3. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism – from biochemistry to genomics. Nat Rev Microbiol. 2005;3(12):937-47. doi: 10.1038/nrmicro1286, PMID 16322742.
4. Larsen TO, Smedsgaard J, Nielsen KF, Hansen ME, Frisvad JC. Phenotypic taxonomy and metabolite profiling in microbial drug discovery. Nat Prod Rep. 2005;22(6):672-95. doi: 10.1039/b404943h, PMID 16311630.
5. Hölker U, Höfer M, Lenz J. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. ApplMicrobiolBiotechnol. 2004;64(2):175-86. doi: 10.1007/s00253-003-1504-3, PMID 14963614.
6. Seviour RJ, Harvey LM, Fazenda M, McNeil B, Archer D, Giavasis I. Microbial production of food ingredients, enzymes, and nutriceuticals. Oxford: Woodhead Publishing; 2013. p. 97-124.
7. Bérdy J. Bioactive Microbial Metabolites: a Personal View. J Antibiot. 2005;58(1):1-26. doi: 10.1038/ja.2005.1.
8. Sanchez S, Demain AL. Bioactive products from fungi. Food Bioactives. 2017:59-87.
9. Brakhage AA, Schroeckh V. Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet Biol. 2011;48(1):15-22. doi: 10.1016/j.fgb.2010.04.004, PMID 20433937.
10. Demain AL, Velasco J, Adrio JL. Industrial mycology: past, present, and future. In: An Z, editor. Handbook of industrial mycology. New York: Marcel Dekker; 2004. p. 1-25.
11. Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, et al. Production of secondary microbial metabolites: regulation by the carbon source. Crit Rev Microbiol. 2010;36(2):146-67. doi: 10.3109/10408410903489576, PMID 20210692.
12. O'Brien J, Wright GD. An ecological perspective of secondary microbial metabolism. CurrOpinBiotechnol. 2011;22(4):552-8. doi: 10.1016/j.copbio.2011.03.010, PMID 21498065.
13. Pettit RK. Small-molecule elicitation of secondary microbial metabolites. MicrobBiotechnol. 2011;4(4):471-8. doi: 10.1111/j.1751-7915.2010.00196.x, PMID 21375710.
14. Xu F, Wu Y, Zhang C, Davis KM, Moon K, Bushin LB, et al. A genetics-free method for high-throughput discovery of cryptic microbial metabolites. Nat Chem Biol. 2019;15(2):161-8. doi: 10.1038/s41589-018-0193-2, PMID 30617293.
15. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75(3):311-35. doi: 10.1021/np200906s, PMID 22316239.
16. Smith E, Lichten CA, Taylor J, MacLure C, Lepetit L, Harte E, et al. Evaluation of the EC action plan against the rising threats from antimicrobial resistance. Rand Corp RB-EC. 2020.
17. Stan D, Enciu AM, Mateescu AL, Ion AC, Brezeanu AC, Stan D, et al. Natural compounds with antimicrobial and Antiviral Effect and Nanocarriers Used for Their Transportation. Front Pharmacol. 2021;12:723233. doi: 10.3389/fphar.2021.723233, PMID 34552489.
18. Chen J, Chen D, Xu Q, Fuhrmann JJ, Li L, Pan G, et al. Organic carbon quality, the composition of main microbial groups, enzyme activities, and temperature sensitivity of soil respiration of an acid paddy soil treated with biochar. BiolFertil Soils. 2019;55(2):185-97. doi: 10.1007/s00374-018-1333-2.
19. Mishra RR. Fungal population concerning temperature and soil moisture. Proc Natl AcadSci U S A, India. 1968;38:211-24.
20. Maphuhla NG, Lewu FB, Oyedeji OO. The effects of physicochemical parameters on analyzed soil enzyme activity from Alice landfill site. Int J Environ Res Public Health. 2020;18(1):221. doi: 10.3390/ijerph18010221, PMID 33396701.
21. Walkley AJ, Black IA. An Examination of the Degtjareff Method for Determining Soil Organic Matter and A

Proposed Modification of the Chromic Acid Titration Method. Soil Sci. 1934;37(1):29-38. doi: 10.1097/00010694-193401000-00003.
22. Allison LE. Organic carbon. In: Black CA, editor. Methods of soil analysis. American Society of Agronomy; 1965. p. 1367-78. doi: 10.2134/agronmonogr9.2.c39.
23. Jackson RM. An investigation of fungi status in Nigerian soil. J Gen Microb. 1958;8:248-58.
24. Waksman SA. Principles of soil Microbiol. Willims Wilkins Co Baltim Md:1-654.
25. Gilman JC. Manual soil fungi. Lowa State College Press; 1957.
26. Ellis MB. DermatiaceusHyphomycetes. Kew Surv UK Common Wealth Mycol Inst;608.
27. Raper KB, Thom C. A manual of the Penicillia. Baltimore: Williams and Wilkins company. Vol. 875.
28. Raper KB, Fennell DI. The genus Aspergillus. Baltimore: Williams & Wilkins Company. p. 685.
29. Vallières C, Avery SV. The Candidate antimalarial drug MMV665909 causes oxygen-dependent mRNA mistranslation and synergizes with quinoline-derived antimalarials. Antimicrob Agents Chemother. 2017;61(9):e00459-17. doi: 10.1128/AAC.00459-17, PMID 28652237.
30. Grycová L, Dostál J, Marek R. Quaternary protoberberine alkaloids. Phytochemistry. 2007;68(2):150-75. doi: 10.1016/j.phytochem.2006.10.004, PMID 17109902.
31. Marek R, Seckarova, Hulova D, Marek J, Dostal J, and Sklenar V. Palmatine. Palmatine and berberine isolation artifacts. J Nat Prod. 2003;66(4):481-6. doi: 10.1021/np0204996, PMID 12713397.
32. Liang D, Xing F, Selvaraj JN, Liu X, Wang L, Hua H, et al. Inhibitory effect of cinnamaldehyde, citral, and eugenol on aflatoxin biosynthetic gene expression and aflatoxin B1 biosynthesis in Aspergillus flavus. J Food Sci. 2015;80(12):M2917-24. doi: 10.1111/1750-3841.13144, PMID 26556681.
33. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar well diffusion method. ActaBiol Med Exp. 1990;15:113-5.
34. Taylor M, Urquhart AJ, Zelzer M, Davies MC, Alexander MR. Picoliter water contact angle measurement on polymers. Langmuir. 2007;23(13):6875-8. doi: 10.1021/la070100j, PMID 17503858.
35. Lawlora K, Knight BP, Barbosa-Jeffersona VL, Laneb PW, Lilleyc AK, Patond GI, et al. Comparison of methods to investigate soil microbial populations under different agricultural management. FEMS Microbiol Ecol. 2000;33(2):129-37. doi: 10.1111/j.1574-6941.2000.tb00735.x, PMID 10967212.
36. Balota EL, ColozziFilho AC, Andrade DS, Dick RP. Long-term tillage and crop rotation affect microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Till Res. 2004;77(2):137-45. doi: 10.1016/j.still.2003.12.003.
37. Gaddeyya G, Shiny NP, Bharathi P, Ratna Kumar PK. Isolation and Identification of oil mycoflora in different crop fields at Salur Mandal. AdvApplSci Res. 2012;3(4):2020-6.
38. Deka HK, Mishra RR. Distribution of soil microflora in jhum fallows in NE India. ActaBotanica Ind. 1984;12:180-4.
39. Kumar PK, Hemanth GP, Shiny N, Samuel K. Isolation and Identification of soil mycoflora in agricultural fields at Tekkali Mandal Srikakulam District. Int. AdvPharmacol. 2015;14(2):484-90.
40. Sharma GPPR. Influence of culture media on growth, colony character, and sporulation of fungi isolated from decaying vegetable wastes. J Yeast Fungal Res. 2010;1(8):157-64.
41. KarmakarPintu, Debbarma P, Das P, Saha AK. Isolation of soil fungi and screening of in vitro antagonistic potentiality of Trichodermaviride against Penicilliumchrysogenum. Eur J Biomed Pharm Sci. 2018;5(2):883-8.
42. Raja M, Praveena G, William SJ. Isolation and Identification of fungi from soil in Loyola College campus, Chennai, India. IntJCurrMicrobiolAppSci. 2017;6(2):1789-95. doi: 10.20546/ijcmas.2017.602.200.
43. Agrios G Noriega Group, editor Mexico. Plant Pathol. 3rd ed. Academic Press; 1988. p. 803.
44. Maheshwari R, Bharadwaj G, Bhat MK. Thermophilic fungi: their physiology and enzymes. MicrobiolMolBiol Rev. 2000;64(3):461-88. doi: 10.1128/MMBR.64.3.461-488.2000, PMID 10974122.
45. Naing TH, Naing TT, Min YY, Yoshimura A. Identification of Fungal isolates from Soils of Rice and Napier Grass. J ExpAgric Int. 2020;42(9):193-203. doi: 10.9734/jeai/2020/v42i930601.
46. Sharma K. Soil mycoflora of Darjeeling tea garden. BIOINFOLET. 2010;7(2):142-3.
47. Christensen EH. A review of fungal ecology. Mycologia. 1989;81(1):5-7.
48. Rai JN, Sharma BB, Agarwal SC. Increased pH tolerance of some Aspergilli isolated from 'Usar' (alkaline) soils. A possible indication of ecological specialization. Sydowia. Ann Mycol. 1970;24:336-44.
49. Phanasenko UT. Ecology of microfungi. Bot Rev. 1967;33:183-225.
50. Jadhav SY, Shinde PP. Isolation and Identification of soil fungi from Kadegaon Tehsil, Sangli District, Maharastra, India. Int. J. Scient. Res Publ. 2017;7(12):616-20.
51. MalleshBaradwad CR. Isolation and Identification of soil mycoflora in Agricultural fields of Hubli Taluk, Karnataka, India. IntJCurrMicrobiolAppSci. 2021;10(8):697-712. doi: 10.20546/ijcmas.2021.1008.079.
52. Chandrashekar MA, SoumyaPai K, Raju NS. Fungal diversity of rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore District, Karnataka, India. Int J CurrMicrobiolAppl Sci. 2014;3(5):559-66.
53. Lei H, Liu A, Hou Q, Zhao Q, Guo J, Wang Z. Diversity patterns of soil microbial communities in the Sophoraflavescens rhizosphere in response to continuous monocropping. BMC Microbiol. 2020;20(1):272. doi: 10.1186/s12866-020-01956-8, PMID 32867674.
54. Ulanowska M, Olas B. Biological properties and prospects for applying for eugenol-a review. Int J Mol Sci. 2021;22(7):3671. doi: 10.3390/ijms22073671, PMID 33916044.
55. Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, et al. Berberine: botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. Front Pharmacol. 2018;9:557. doi: 10.3389/fphar.2018.00557, PMID 30186157.

56. Song D, Hao J, Fan D. Biological properties and clinical applications of Berberine. Front Med. 2020;14(5):564-82. doi: 10.1007/s11684-019-0724-6, PMID 32335802.
57. Jenks JD, Hoenigl M. Treatment of aspergillosis. J Fungi (Basel). 2018;4(3):94. doi: 10.3390/jof4030098, PMID 30126229.
58. Baell JB. Feeling nature's PAINS: natural products, natural product drugs, and pan assay interference compounds (PAINS). J Nat Prod. 2016;79(3):616-28. doi: 10.1021/acs.jnatprod.5b00947, PMID 26900761.
59. Bisson J, McAlpine JB, Friesen JB, Chen SN, Graham J, Pauli GF. Can invalid bio-actives undermine natural product-based drug discovery? J Med Chem. 2016;59(5):1671-90. doi: 10.1021/acs.jmedchem.5b01009, PMID 26505758.
60. Abu-Salah KM. Amphotericin B: an update. Br J Biomed Sci. 1996;53(2):122-33. PMID 8757689.
61. Tevyashova AN, Olsufyeva EN, Solovieva SE, Printsevskaya SS, Reznikova MI, Trenin AS, et al. Structure-antifungal activity relationships of polyene antibiotics of the amphotericin B group. Antimicrob Agents Chemother. 2013;57(8):3815-22. doi: 10.1128/AAC.00270-13, PMID 23716057.
62. Zida A, Bamba S, Yacouba A, Ouedraogo-Traore R, Guiguemdé RT. Anti-Candidaalbicans natural products, sources of new antifungal drugs: a review. J Mycol Med. 2017;27(1):1-19. doi: 10.1016/j.mycmed.2016.10.002, PMID 27842800.
63. Xie Y, Liu X, Zhou P. In-vitro antifungal effects of Berberine against Candida sp Planktonic and Biofilm Conditions. Drug Ther. 2020;14:87-101.
64. Li DD, Xu Y, Zhang DZ, Quan H, Mylonakis E, Hu DD et al. Fluconazole assists berberine to kill fluconazole-resistant Candida albicans. Antimicrob Agents Chemother. 2013;57(12):6016-27. doi: 10.1128/AAC.00499-13. PMID 24060867.
65. Xu Y, Quan H, Wang Y, Zhong H, Sun J, Xu J et al. Requirement for ergosterol in berberine tolerance underlies synergism of Fluconazole and Berberine against fluconazole-resistant Candida albicans Isolates. Front Cell Infect Microbiol. 2017;7:491. doi: 10.3389/fcimb.2017.00491, PMID 29238700.
66. Han Y, Lee JH. Berberine synergy with amphotericin B against disseminated candidiasis in mice. Biol Pharm Bull. 2005;28(3):541-4. doi: 10.1248/bpb.28.541, PMID 15744087.
67. Lam P, Kok SH, Lee KK, Lam KH, Hau DK, Wong WY et al. Sensitization of Candida albicans to terbinafine by berberine and berberrubine. Biomed Rep. 2016;4(4):449-52. doi: 10.3892/br.2016.608, PMID 27073630.
68. Ahmad N, Alam MK, Shehbaz A, Khan A, Mannan A, Hakim SR, et al. Antimicrobial activity of clove oil and its potential in treating vaginal candidiasis. J Drug Target. 2005;13(10):555-61. doi: 10.1080/10611860500422958, PMID 16390816.
69. Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in the essential oil of the Syzygiumaromaticum (L.) by extraction, purification, and analysis of its main component, eugenol. Braz J Microbiol. 2011;42(4):1269-77. doi: 10.1590/S1517-83822011000400004, PMID 24031751.