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Original Research Article

OPTIMIZATION OF CULTURE CONDITIONS FOR IMPROVED GROWTH AND PRODUCTION OF ANTIMICROBIAL METABOLITES BY ENDOPHYTIC FUNGUS, *Colletotrichum gloeosporioides* STRAIN LCM 938.01 ISOLATED FROM *Synclisia scabrida* MIERS

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ABSTRACT

An ethnomedical plant, Synclisia scabrida Miers, has been reported to host endophytic fungi that synthesize secondary compounds with strong antimicrobial properties. This work aimed to optimize cultural conditions for the growth and production of antimicrobial metabolites by the endophytic fungus *Colletotrichum gloeosporioides* strain LCM 938.01 isolated from the leaves of *Synclisia scabrida* Miers. The identity of the fungus was confirmed using molecular techniques. The effects of different temperature conditions, culture media, incubation time, and carbon and nitrogen sources were evaluated by measuring inhibition zone diameters against five pathogenic bacteria and a fungus using the agar well diffusion method. Optimal growth and production of antimicrobial compounds were observed when the organism was cultured on rice medium at 25 °C, with the incorporation of 1 % fructose and glycine, which serve as carbon and nitrogen sources, respectively, into the growth medium, and incubated for 20 days. Significant growth and synthesis of metabolites with antimicrobial properties were observed under these optimal conditions compared with the basal media, which served as the control. This work has shown the need to optimize cultural conditions for maximum growth and production of antimicrobial metabolites by the endophytic fungus *Colletotrichum gloeosporioides* strain LCM 938.01. It will also guide further research on growth conditions for the characterization and isolation of antimicrobial compounds from this endophytic fungus for both pharmaceutical and agricultural purposes.

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INTRODUCTION

In medical practice, the need to search for other means of acquiring bioactive compounds, other than plants, for use as lead compounds in the development of new drugs is of utmost important. This is as a result of an increase in the emergence of multiple drug-resistant pathogens which have led researchers to search for novel antimicrobial

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compounds. Antimicrobial resistance has been reported to be among the greatest global concerns in the 21st century because of the fast increase in antimicrobial resistant infections [1]. The shrub *Synclisia scabrida Mier*s, sometimes referred to as "goat weed," grows throughout tropical Africa, particularly in Southern Nigeria, Cameroon, and some other African countries. Traditional medicine practitioners use this plant to address conditions like gastrointestinal issues, dyspepsia, menstrual discomfort, sexually transmitted diseases, and gastric ulcers. The roots, stem, and leaves are believed to possess medicinal qualities [2, 3]. The ethanolic and aqueous root extracts of *Synclisia scabrida* Miers was shown to possess phytocompounds such as alkaloids, flavonoids, tannins, cyanogenic glycosides, terpenins, proteins, saponins and lipids which have been shown to be accountable for the different pharmacological effects observed in the plant [4, 5].

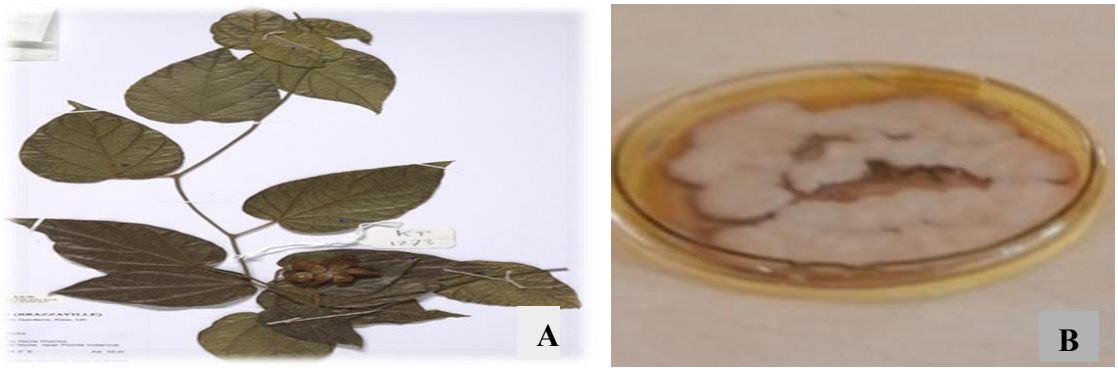


Figure 1: A: *Synclisia scabrida* Miers (Leaves and stem); B: Culture plate of *Colletotrichum gloeosporioides* strain LCM 938.01

Endophytic fungi represent those fungal organisms that reside in the internal tissues of plants and causes no harm to the plant host [6, 7]. They produce a wide range of secondary metabolites within the plant host and have been described as the nature's hidden source of phytocompounds, with varying pharmacological effects such as antimicrobials, antioxidants, insecticides, herbicides etc. [8, 9]. Numerous bioactive phytochemical compounds such as cryptocandin, taxol kakadumucins, subglutinols A and B, etc. have been discovered from endophytic fungi and characterized for their ability to exhibit cytotoxic, antidiabetic, antimicrobial, and anticancer activities [10]. *Synclisia scabrida* Miers is a host of diverse endophytic fungi including *Colletotrichum gloeosporioides* strain LCM 938.01. The production of these bioactive compounds have been found to be affected by the cultural conditions such as the temperature of incubation, culture media, incubation time, P^h, carbon and nitrogen sources etc. used for the growth of these endophytic fungi [9, 11].

Colletotrichum gloeosporioides strain LCM 938.01 is an endophytic fungus, isolated from the leaf of *synclisia scabrida* Miers. The specie, *Colletotrichum gloeosporioides,* is an important facultative plant pathogen belonging to the family Phyllachoraceae of the division Ascomycota which causes anthracnose disease in a wide range of crops. It causes post-harvest infections in about 470 different host genera [12] and some strains that exhibit biotrophic life styles live in endophytic association with other plants including *S. scabrida* Miers where they cause no sign of infection [13, 14]. It has been isolated from many plants including *Plumeria acutifolia*, *Synclisia scabrida* Miers and *Calotropis gigantea* [15, 16, 17]. *Colletotrichum gloeosporioides* strain LCM 938.01 produce a wide range of secondary metabolites which include flavonoids, tannins, saponins, alkaloids, cyanogenic glycosides, etc. with potent antimicrobial activities [16] Further characterization of secondary metabolites produced by *Colletotrichum gloeosporioides* revealed phytotoxic activity due to the presence of taxol, a potent anticancer compound and extracellular enzymes [18]. Despite these potentials, the cultural conditions required for optimal growth and maximum production of bioactive compounds by *Colletotrichum gloeosporioides* strain LCM 938.01 *have* not been properly studied. Hence, the need to study the cultural conditions for growth and synthesis of antimicrobial metabolites by the endophytic fungus, *Colletotrichum gloeosporioides* strain LCM 938.01. The best generation of bioactive metabolites by various microbial strains depends on the maintenance and optimization of appropriate growth conditions.

MATERIALS AND METHODS

Test Microorganisms

The test organisms used in this study were five pathogenic bacteria which included *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and a fungus*, Aspergillus niger*. These were lab cultures that had a known susceptibility profile to popular antibiotics. These were lab cultures with established susceptibility to popular antibiotics.

Optimization of cultural conditions of *Colletotrichum gloeosporioides* strain LCM 938.01 for growth and synthesis of antibiotic compounds

In this study, different cultural conditions, which may affect the growth and generation of antibiotic compounds responsible for the observed antimicrobial activity exhibited by this endophytic fungus, according to [16] were optimized. These conditions include the incubation temperature, culture media, carbon and nitrogen sources, and the length of incubation period and were evaluated by varying the cultural conditions of growth of the fungus while not altering the make-up of the basal medium. The method of [19] was adopted with slight modification. Fungal growth was measured as mycelial dry weight while production of antimicrobial metabolites was measured as inhibition zone diameter against the test organisms. The media and chemicals used in this study were products of High-Media, Mumbai, India.

Preparation of basal medium

The basal medium used was potato dextrose broth because it is one of the most commonly used medium on a laboratory scale for growth of fungi. After sterilizing, a 250 ml conical flask with 100 ml of broth was allowed to cool. It was inoculated with 5 mm diameter of 7-day-old culture of *Colletotrichum gloeosporioides* strain LCM 938.01 and incubated at room temperature for 14 days. The mycelia produced was removed from the medium using Whatman filter paper, washed with sterile distilled water, dried at 50 ^{o}C and the dry weight (mg/100ml) measured. The metabolite was extracted from the supernatant with 25 ml of ethyl acetate. The ethyl acetate extract was separated from the media using a separating funnel and allowed to dry completely to obtain the fungal metabolite. The synthesis of antibiotic compounds was evaluated by measuring the inhibition zone diameter produced by the fungal metabolites against the test organisms using agar diffusion method.

Variation of incubation temperature on the growth and synthesis of antibiotic compounds

To determine the optimal temperature requirement for fungal growth and synthesis of antibiotic compounds, a temperature range of 20-40 ^{o}C and room temperature for the basal medium was used. A total of 100 ml of the basal medium was sterilized at 121 °C for 20 min in a 250 ml conical flask. Under aseptic condition, 5 mm diameter of 7-day old culture of *Colletotrichum gloeosporioides* strain LCM 938.01 was inoculated into the basal medium and, then incubated at the specific temperature conditions for 14 days. The fungal mycelia were removed, dried and weight (biomass yield) recorded. The fungal metabolites were tested for antimicrobial activity and the zones of inhibition recorded.

Variation of culture media on the growth and synthesis of antibiotic compounds

This was done in order to select the optimal growth medium suitable for the growth of *Colletotrichum gloeosporioides* strain LCM 938.01. Sabouraud dextrose broth, malt extract broth, potato dextrose broth and rice medium were used. Potato dextrose broth (PDB), malt extract broth (MEB) and Sabouraud dextrose broth (SDB) were prepared according to the manufacturers' standard method of preparation. Rice medium was prepared by weighing 10 g of local rice into 100 ml conical flasks. A 30 ml volume of distilled water was transferred into the flasks, sealed and sterilized using an autoclave at 121 °C for 15 min and allowed to cool down completely. Thereafter, the conical flasks containing PDB, MEB, SDB and rice medium were inoculated with 5 mm mycelia disks of seven-day old culture of *Colletotrichum gloeosporioides* strain LCM 938.01 under aseptic condition and incubated for 14 days at room temperature. The fungal mycelia were removed, dried and weight (biomass yield) recorded. The fungal metabolites were tested for antimicrobial activity and the zones of inhibition recorded.

Variation of carbon and nitrogen sources on the growth and synthesis of antibiotic compounds

This study included glucose, fructose, lactose, sodium citrate, and sucrose as carbon sources, and nitrogen sources comprised arginine, glycine, sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl), and potassium nitrate (KNO_3). To test the impact of various carbon and nitrogen sources on endophytic fungal growth, 100 ml of basal media in a 250 ml Erlenmeyer flask was mixed with 1% w/v of each. Each flask with 1% w/v carbon sources was infected with a 5 mm of 7-day-old culture of Colletotrichum gloeosporioides strain LCM 938.01 under aseptic conditions and incubated for 14 days at room temperature. The fungal mycelia were

removed, dried and the weight (biomass yield) recorded. The fungal metabolites were tested for antimicrobial activity and the zones of inhibition recorded.

Variation of incubation time on the growth and synthesis of antibiotic compounds

The length of incubation which ranged from 5 to 25 days was used to determine the effect of incubation time on the growth and production of antimicrobial metabolites by the endophytic fungus, *Colletotrichum gloeosporioides* strain LCM 938.01.

To achieve this, sterile 100 ml volume of the basal medium in 250 ml conical flasks were inoculated with a 5 mm mycelial disk of a 7-day-old endophytic fungal culture under aseptic conditions and incubated for 25 days at room temperature. At 5-day intervals, the fungal mycelia were removed, dried, and the weight recorded. The fungal metabolites resulting from each flask was tested for antimicrobial activity, and the zones of inhibition were recorded.

Statistical analysis

All experiments were carried out in triplicate, and the results obtained were expressed as mean ± standard error of the mean (SEM) of sample replicates (n=3). Statistical Package for the Social Sciences (SPSS-20 for Windows) was the software used for data analysis. Graphical presentation of parameters was done using Microsoft Excel 2016.

RESULTS

Variation of cultural conditions for optimal growth and synthesis of antibiotic compounds

Optimizing the physical conditions of growth of an organism can significantly boost the growth and yield of antimicrobial compounds produced by the organisms. This is because the biosynthesis of antibiotic compound is a property of microorganisms which depends significantly on the cultural conditions of growth. Consequently, the nutritional and physical conditions of the growth culture can be adjusted to foster the growth and production of antimicrobial metabolites (Sezhian & Bose, 2019)[20] [20]. The ability of an endophyte to produce bioactive metabolites is an important factor in endophyte-plant relationship. Endophytic fungi produce notable secondary metabolites, including antimicrobial compounds, which is crucial in the symbiotic relationship between the endophytic fungi and the host plant [21]. The production of specific secondary metabolites such as alkaloids, tannins, flavonoids, glucosides etc. by endophytic fungi can directly impact on the inhibition zone diameter thereby reducing the minimum inhibitory concentration required to exert therapeutic effect [22]. These findings have shown that the endophytic fungus, *Colletotrichum gloeosporioides* strain LCM 938.01 with the Genbank Accession Number: grows optimally on rice medium.

Variation of temperature on the growth and synthesis of antibiotic compounds

In order to prevent excessive production, regulatory systems within the cell might occasionally restrict the synthesis of bioactive secondary metabolites in microorganisms. However, these pathways can occasionally lead to undesirable low levels of synthesis, which can be significantly raised by optimizing physical (temperature, salinity, pH, and light) and chemical (media components, precursors, and inhibitors) parameters for microbial development [23].

This study has investigated the influence of cultural parameters on the proliferation and synthesis of antimicrobial substances by the endophytic fungus, *Colletotrichum gloeosporioides* strain LCM 938.01. Figures 2A and B illustrate how temperature affects the growth and formation of bioactive compounds by the fungus. The influence of temperature on the growth and bioactive metabolite production of this fungus are presented in Figure 2A and B. Highest biomass (1351 mg/100ml) and antimicrobial metabolite yield (IZD = 20 ± 0.0 mm against *Bacillus subtilis*) was obtained at 25 °C, followed by 30 °C with the biomass production of 998 mg/100ml and IZD of 16 ± 0.16 mm and 15 ± 0.0 mm against *B. subtilis* and *E. coli* respectively. However, there was no production of antimicrobial metabolites active against *P. aeruginosa* at 30 °C. The antimicrobial metabolites active against *P. aeruginosa* were produced at 25 °C (IZD= 12 ± 0.33 mm) and 35 °C (IZD = 8 ±0.16 mm). The lowest antimicrobial production was recorded at 40 °C against *B. subtilis* (IZD = 6 ± 0.33 mm) and A. niger (IZD = 8 ± 0.0 mm) only, with the biomass of 524 mg/100ml. The antimicrobial metabolite against *K. pneumoniae* was produced at 20 °C and 30 °C (IZD = 10 ± 0.16 mm). The synthesis of antimicrobial metabolites gradually decreased as the temperature increased from 25 to 40 °C.

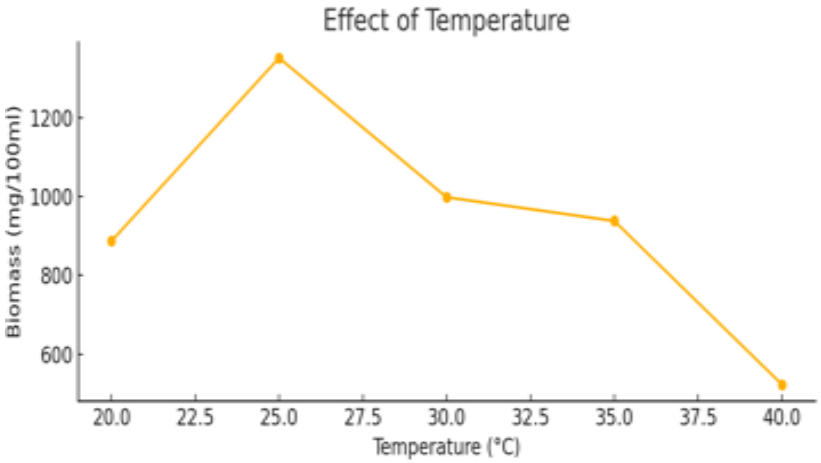


Figure 2A: The effect of temperature variations on the growth (biomass) of *Colletotrichum gloeosporioides* strain LCM 938.01.

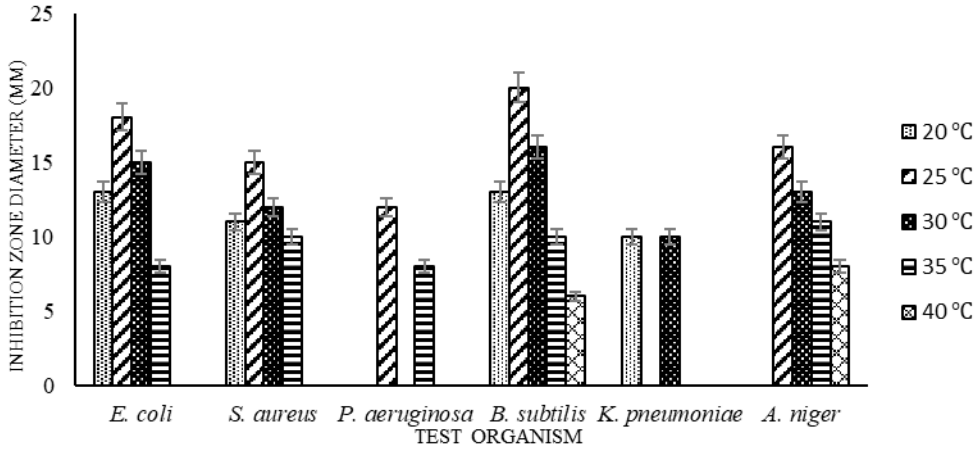


Figure 2B. The effect of temperature variations on the production of antimicrobial metabolite by *Colletotrichum gloeosporioides* strain LCM 938.01. *E. coli* = *Escherichia coli; S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *B. subtilis* = *Bacillus subtilis*; *K. pneumoniae* = *Klebsiella pneumoniae* and *A. niger* = *Aspergillus niger*

Effect of culture media variation on the growth and antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01

Parameter optimization is one of the key parts of microbial production system. Optimum culture condition imparts suitable physiological and physical conditions that would affect the antimicrobial agent production of the selected strain [24]. The effects of different culture media on growth and production of antimicrobial metabolites of *Colletotrichum gloeosporioides* strain LCM 938.01are shown in Figures 3A and 3B. Maximum production of antimicrobial metabolites was observed in rice medium (IZD = 26 ± 0.0 mm against *K. pnemoniae*) followed by SDB (IZD = 21 ± 0.0 mm against *Staph. aureus*) with biomass production of 322 mg/100ml. The growth of *Colletotrichum gloeosporioides* strain LCM 938.01 in rice medium led to the production of metabolite which showed antimicrobial activity against all the test organisms used in this study but the biomass yield could not be determined. Growth of the fungus in PDB produced a biomass of 320 mg/100 ml and showed activity against *Staph. aureus* (IZD = 11 ±0.16 mm); growth in SDB produced a biomass of 322 mg/100ml and metabolite with highest antimicrobial activity against *Staph aureus* (IZD = 21 ± 0.0 mm) while growth in malt extract medium produced antimicrobial metabolite active against *B. subtilis* (IZD = 17 ± 0.16 mm). *E. coli* and *K pneumoniae* were not sensitive to the metabolite produced by the endophytic fungus in

PDB, SDB and MEB. However, the antimicrobial metabolite produced in all the culture media studied was not active on the fungal test organism, *A. niger*. The growth of *Colletotrichum gloeosporioides* strain LCM 938.01 in rice medium produced metabolite which showed higher zones of inhibition than when the other media were used. This is an indication that the ingredients of rice medium promoted the production of bioactive metabolite by the fungal isolates.

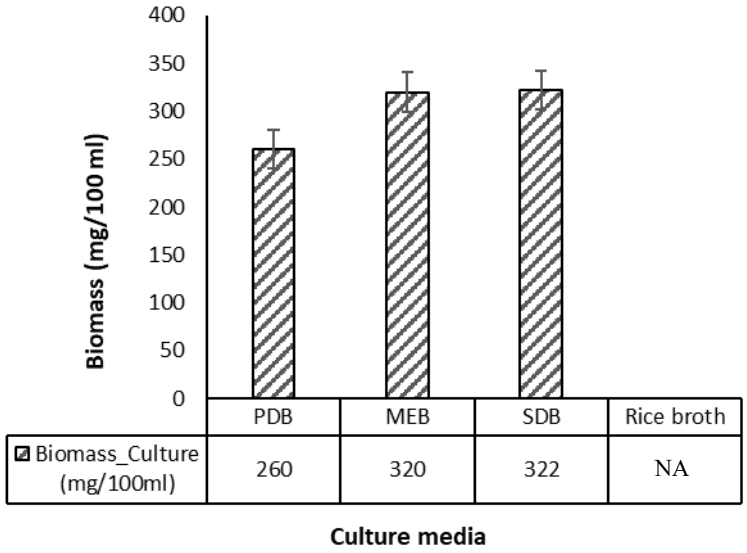


Figure 3A: Effect of culture media variation on biomass of *Colletotrichum gloeosporioides* strain LCM 938.01. NA: Not applicable

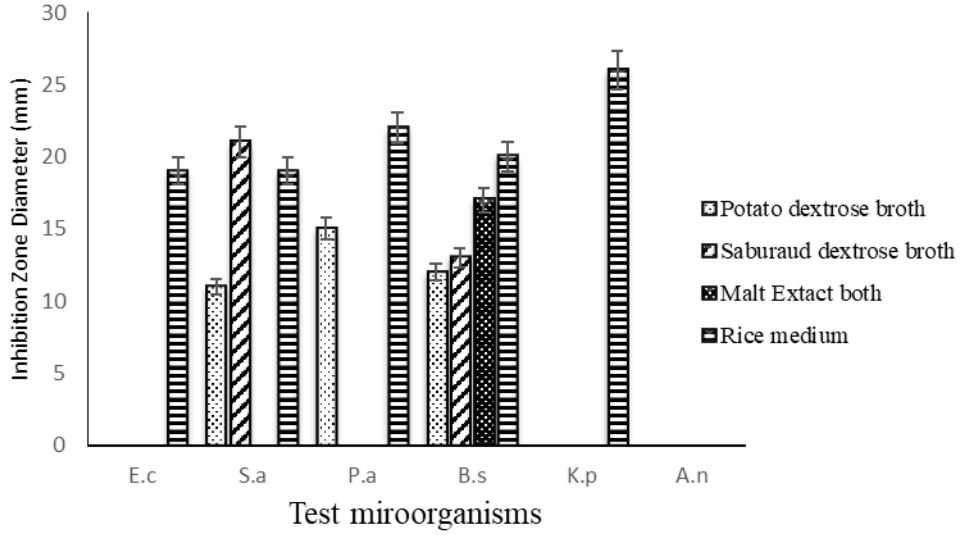


Figure 3B: Effect of culture media variation on antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; B.s = *Bacillus subtilis*; K. p = *Klebsiella pneumoniae* and A. n = *Aspergillus niger*

Effect of carbon sources on the growth and production of antimicrobial metabolite by the fungus, *Colletotrichum gloeosporioides* strain LCM 938.01

Figures 4A and 4B illustrate the effects of various carbon sources on the growth and antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. In this study, the fungus was cultured in basal medium, with the original carbon source replaced by each of the carbon sources being evaluated. Fructose had maximum influence on growth (414 mg/100 ml) and production of antimicrobial metabolite active against the test organisms (Figure 4A and 4B). The addition of fructose into the growth medium of the fungi resulted in the production of metabolite active against the test organism except *P. aeruginosa*, with highest activity against *A. niger* (IZD = 41 ± 0.33

mm). The production of antimicrobial metabolite against *P. aeruginosa* was enhanced by incorporating lactose and sucrose (IZD = 12 ± 0.0 mm and 15 ± 0.0 mm respectively) into the growth medium. The addition of sucrose into the growth medium produced metabolite active on all the test organisms. Inclusion of lactose resulted in the production of metabolite active against *P. aeruginosa* (IZD= 12 ± 0.0 mm), *B. subtilis* (IZD = 12 ± 0.0 mm) and *A. niger* (IZD= 11 ± 0.16mm). The zones of inhibition against *A. niger* recorded for fructose and sucrose respectively were 41.47 ± 0.63 mm and 19.69 ± 0.2 mm.

Bhattacharyya and Jha [12] reported that sucrose and asparagine were the most suitable carbon and nitrogen sources, respectively, for maximizing bioactive metabolite production by *Aspergillus* strain TSF 146. Similarly, the optimized growth and metabolite production of *Fusarium solani* using dextrose as the carbon source and yeast extract as the nitrogen source in the basal medium [25]. In the present study, however, fructose was the most effective carbon source for antimicrobial metabolite production by *Colletotrichum gloeosporioides* strain LCM 938.01 against the test organisms, followed by sucrose. These results are consistent with other findings [26]; [26].

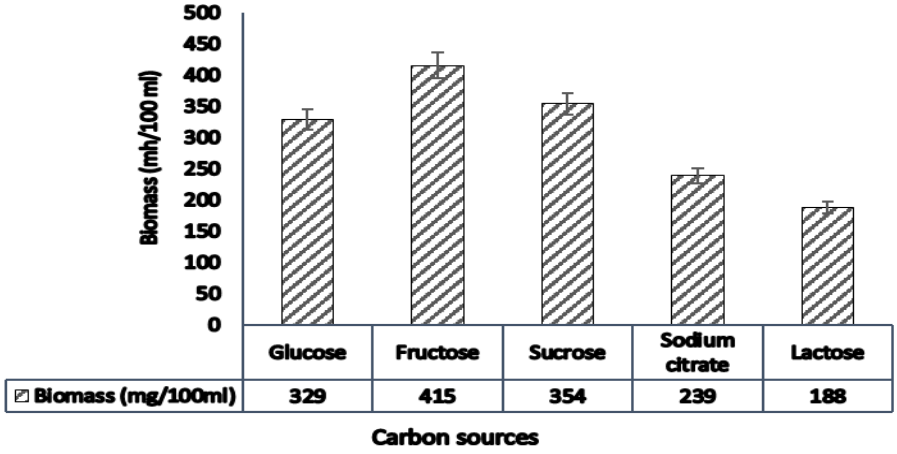


Figure 4A: Effect of different carbon sources on the growth (biomass) of *Colletotrichum gloeosporioides* strain LCM 938.01

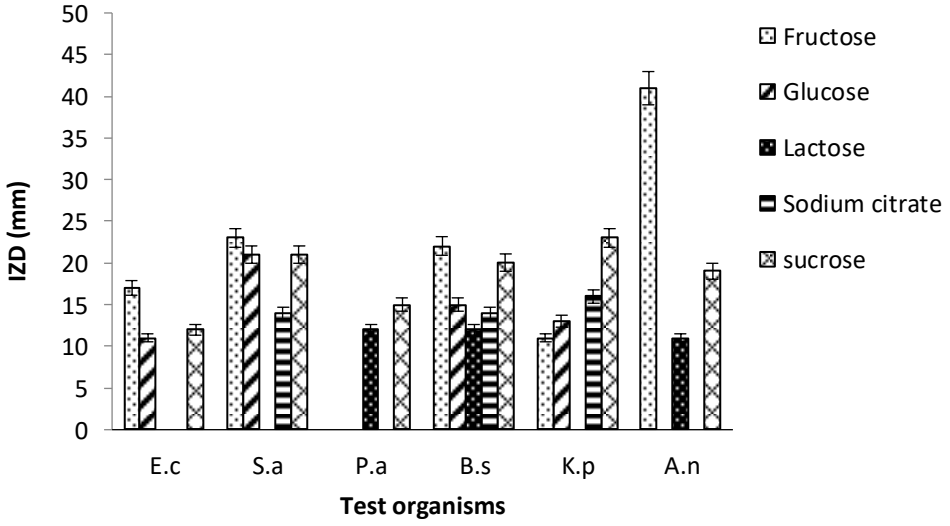


Figure 4B: Effect of different carbon sources on the antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. E. c = *Escherichia coli;* S. a =*Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; B. s = *Bacillus subtilis*; K. p = *Klebsiella pneumoniae* and A. n = *Aspergillus niger*

Influence of Incubation Time on Biomass Yield and Antimicrobial Metabolite Production of *Colletotrichum gloeosporioides* Strain LCM 938.01

Figures 5A and 5B illustrate the effects of various nitrogen sources on the growth and antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. Among the N-sources used for cultivation of the fungus, glycine was the most effective with biomass yield of 368 mg/100 ml and IZD of 23 ± 0.33 mm against *S. aureus*). This was followed by potassium nitrate (Biomass: 274 mg/100 ml; IZD: 19 ± 0.58 mm against *B. subtilis*) for maximum antimicrobial metabolite production. Sodium nitrate (biomass: 249 mg/100 ml) produced antimicrobial metabolites active against *E. coli* and *P. aeruginosa* (IZD = 12 ± 0.33 mm). Ammonium chloride, with biomass yield of 269 mg/100 ml produced metabolites with activity against *S. aureus* (IZD: 11.0 ± 0.58), B. subtilis (IZD: 14.0 ± 0.0) and the fungus, A. niger (IZD: 12.0±0.33), thereby showing activity against both Gram positive bacteria and fungus. Arginine, with biomass of 250 mg/100 ml, was the nitrogen source with activity against only one test organism, *P aeruginosa*, with IZD of 12.0 ± 0.0. Yeast extract was reported to be the best organic nitrogen sources for biomass production by the fungus, *Athelia rolfsii* [27]

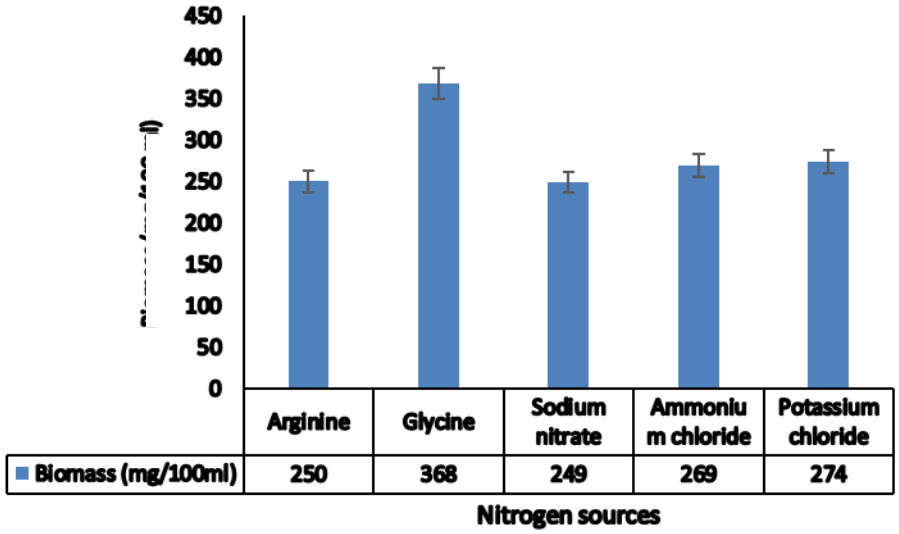


Figure 5A: Effect of different nitrogen sources on growth (biomass) of *Colletotrichum gloeosporioides* strain LCM 938.01

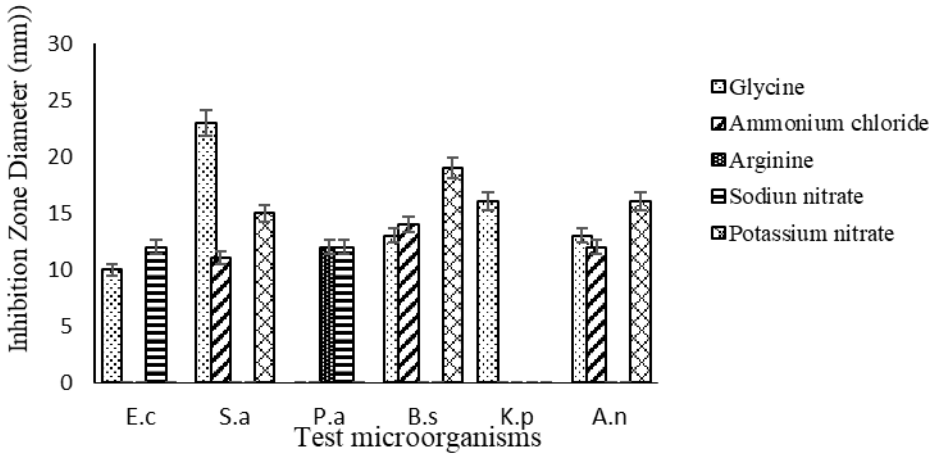


Figure 5B: Effect of different nitrogen sources on antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; B. s = *Bacillus subtilis*; K. p = *Klebsiella pneumoniae* and A. n = *Aspergillus niger*

Effect of incubation time variation on growth (biomass yield) and antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01

Figures 6A and 6B illustrate the influence of incubation time on the growth and antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01.

Figure 6A showed that there was steady increase in fungal biomass as the incubation time was progressed from 5 to 20 days, with the highest production at day 20 (biomass: 230 mg/100 ml). Beyond 20 days (Day 25), there was a decline in biomass production. Similarly, there was gradual increase in the production of antimicrobial metabolites active against all the test organisms used in this study except *P. aeruginosa* as the incubation period was extended, with maximum production observed at day 20. The findings indicated that a 20-day incubation period under stationary conditions was optimal for producing antimicrobial metabolites active against the bacterial and fungal test organisms (Figures 6A and 6B). Incubation beyond 20 days resulted in a decline in the antimicrobial activity of the bioactive secondary metabolites

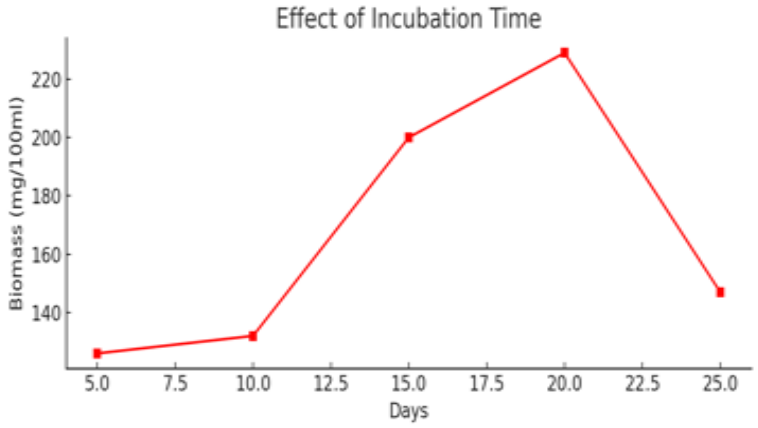


Figure 6A: Effect of incubation time variation on the growth (biomass) of *Colletotrichum gloeosporioides* strain LCM 938.01

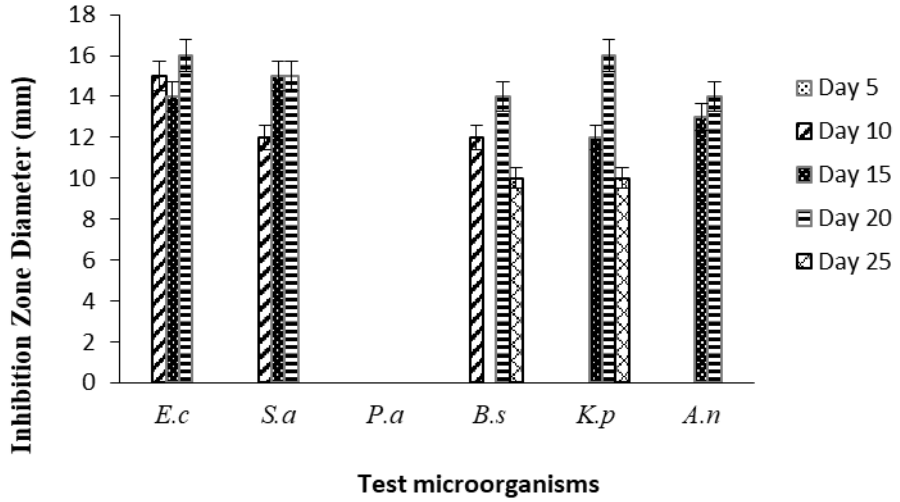


Figure 6B: Effect of incubation time variation on antimicrobial metabolite production of *Colletotrichum gloeosporioides* strain LCM 938.01. E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; B. s = *Bacillus subtilis*; K. p = *Klebsiella pneumoniae* and A. n = *Aspergillus niger*

DISCUSSION

The ability of *Colletotrichum gloeosporioides* strain LCM 938.01 to grow optimally and maximize its antimicrobial production depends highly on the physical growth factors such as the temperature [23]. The highest fungal growth and antimicrobial compound production occurred at 25°C, with a biomass yield of 1,351 mg/100 mL and an inhibition zone diameter (IZD) of 20 ± 0.0 mm against *Bacillus subtilis*. This was followed by 30°C, which produced a biomass yield of 998 mg/100 mL and IZD values of 16 ± 0.16 mm against *B. subtilis* and 15 ± 0.0 mm against *Escherichia coli* (Figures 2A and 2B). This temperature requirement is common to many fungal species and shows that the fungus, *Colletotrichum gloeosporioides* strain LCM 938.01 is a mesophic organism and grows well at moderate temperature condition. The optimal temperature for enhanced production of bioactive compounds in endophytic *Geosmithia pallida* (KU693285) isolated from *Brucea mollis* Wall ex. Kurz was found to be 25 to 30 °C [28]. At 25 °C, *G. pallida* produced maximum metabolite (inhibition zone 25.33 ± 0.61 mm).These results support the findings which showed that the production of secondary metabolites in fungi depends on temperature with optimal yields at the temperature which supports growth and high metabolic activities [29]. Reduced growth and production of antimicrobial compounds were observed at the extreme temperature conditions. At lower temperatures, that is, below 25 ℃, there is a decline in the growth and antimicrobial metabolite production by the fungus (Biomass = 780 mg/100 ml; IZD = 13 ± 0.3 mm against both *B. subtilis* and *Aspergillus niger*). This could be as a result of a slowdown in enzyme activities and the rates of metabolism, thereby reducing the synthesis of antimicrobial compounds. The lowest growth and antimicrobial production was recorded at 40 °C (Biomass = 524 mg/100 ml; IZD = 6.0 ± 0.33 mm and 8.0 ± 0.0 against *B. subtilis* and A. niger respectively). The rise in temperature may exert a thermal stress on the fungus, thereby resulting in reduced growth and antimicrobial metabolite production [30]. This similar pattern of temperature dependence have been reported for some other antifungal and antibacterial producers such as Penicillium spp which show maximum antibiotic production at 25-28 °C [31]. The results of this study follows this pattern, thereby emphacizing the need for optimizing the growth temperature of *Colletotrichum gloeosporioides* strain LCM 938.01 for industrial application.

Rice medium is a suitable alternative for cultivating endophytic fungi because its high carbohydrate content supports the growth of filamentous fungi [32]. It is also readily available, affordable, and provides an energy source for microorganisms, including fungi. Similar work reported that metabolites produced by *Penicillium citrinum* E-346 exhibited the highest antibacterial activity when the fungus was cultivated on rice medium [33].

This study supports these findings, as the maximum production of antimicrobial metabolites by the *Colletotrichum gloeosporioides* strain LCM 938.01 was observed in rice medium, followed by SDB, MEB, and PDB 9 (Figures 3A and 3B). Maximum growth and metabolite production by *Aspergillus niger* (MTTC 961) was found to be sabouraud dextrose broth [34]. However, Potato dextrose broth medium at 25 °C was optimized for maximum growth and production of antimicrobial metabolites in *Aspergillus terreus* KC 582297 and Aspergillus strain TSF 146 [20]; [12]. Similarly, optimized culture media was determined as Potato Dextrose Broth (PDB) using dextrose as its carbon source, yeast extract as its nitrogen source along with 5g/l NaCl concentration at 30 ℃ in pH 5, for the maximum growth and production of bioactive compounds of *Aspergillus tamari* [20].

Kalyani [34] reported that *Aspergillus niger* MTTC 961 achieved its highest growth and metabolite production in Sabouraud dextrose broth. In contrast, potato dextrose broth incubated at 25°C was found to be the optimal medium for maximizing the growth and antimicrobial metabolite production of *Aspergillus terreus* KC 582297 and *Aspergillus* strain TSF 146, respectively [20];[12] . Similarly, potato dextrose broth supplemented with dextrose as the carbon source, yeast extract as the nitrogen source, and 5 g/L NaCl, at 30°C and pH 5, provided the optimal conditions for the growth and bioactive compound production of *Aspergillus tamari* [20]. These findings has shown that the growth medium play a critical role in maintaining optimal growth and ability to maximize the production of antimicrobial compounds by the fungus, *Colletotrichum gloeosporioides* strain LCM 938.01.

Carbon sources are group of important nutrients which greatly affect the metabolism, morphology and production of secondary metabolites in microorganisms. This work evaluated the effects of five carbon sources—glucose, fructose, sucrose, sodium citrate, and lactose—on the growth (biomass yield) and production of antimicrobial metabolites (measured as inhibition zone diameter, IZD) of *Colletotrichum gloeosporioides* strain LCM 938.01, an endophytic fungus with known antimicrobial potential [16]. The growth of this organism showed that fructose yielded the highest biomass (415 mg/100 ml), with the highest production of

antimicrobial metabolites with highest IZD values across the most test organisms, especially against *A. niger* (IZD = 41.0 ± 0.33) and *staph aureus* (IZD = 23.0 ± 0.0). This was followed by sucrose and glucose (biomass: 354 mg/100 ml and 329 mg/100 ml respectively), which supported moderate activity with the production of antimicrobial metabolites active against *K. pneumoniae* (IZD = 23.0 ± 0.16) and *staph. aureus* (IZD = 21.0 ± 0.16) respectively. This agrees with the report of Gomaa [35] that *Alternaria alternata* produce maximum secondary metabolites with fructose. The high biomass yield observed with sucrose is consistent with other findings [36]; [37]. These works showed that sucrose supported the highest biomass in *Trichoderma harzianum* and *Fusarium solani*, respectively, thereby highlighting the strain-specific nature of carbon-source optimization. In contrast, it was established that optimal biomass production of an endophytic fungus, *Athelia rolfsii* Strain orchid by supplementation with glucose, yeast, SDB and a temperature of 29 °C [27]. Fructose and glucose supported moderate antimicrobial activity. Similar trends were reported in other fungi, showing that these sugars are effective but less potent than sucrose[38].

Sodium citrate and lactose supported relatively lower growth (239 mg/100 ml and 188 mg/100 ml respectively), with antimicrobial metabolites active against *K. pneumoniae* (IZD = 16.0 ± 0.0) and *P. aeruginosa* and *B. subtilis* (IZD = 12.0 ± 0.33) respectively. This suggests that monosaccharides, particularly fructose, are more efficiently utilized by this fungal strain for growth and production of bioactive compounds effective against fungi than disaccharides or organic acids. This might be as a result of the ability of fructose to enhance carbon flow through glycolysis and the Tricarboxillic Acid cycle, thereby providing the necessary precursors for biosynthesis and energy [39]. These findings align with studies showing that high-energy sugars such as sucrose often trigger secondary metabolite biosynthesis in fungi. These findings confirm that carbon sources not only serve as nutrients but also act as switches that activate biosynthetic gene clusters, thereby influencing antimicrobial metabolite production.

Nitrogen is a critical element for microbial growth and secondary metabolite biosynthesis. It serves as a building block for proteins, nucleic acids, and enzymatic cofactors, and it modulates biosynthetic pathways linked to antimicrobial compound production. The nitrogen source type (organic or inorganic) can significantly influence both fungal biomass and secondary metabolism [37].

This study showed that incorporating glycine into the growth medium of *Colletotrichum gloeosporioides* LCM 938.01 resulted in increased biomass (368 mg/100 ml) and antimicrobial metabolites active against all test organisms, including *A. niger* (a fungus), except *P. aeruginosa*, with the highest activity against *Staph. aureus* (IZD = 23.0 ± 0.33. Patel (2020)[37] [37] reported that glycine supported the highest biomass and moderate antifungal compound production in *Fusarium solani*, indicating its suitability for fungal growth. Ammonium chloride supported moderate growth of the fungus, with metabolites active against *Staph. aureu*, *B. subtilis,* and *A. niger*, with the highest activity against *B. subtilis* (IZD = 23.0 ± 0.33). This supports the findings which reported that ammonium nitrate affected both growth and secondary metabolism in Aspergillus nidulans [29]. Kumar [40] noted that ammonium sulfate enhanced growth but repressed secondary metabolites in Penicillium citrinum. El-Bondkly [38] found that arginine supported moderate growth but low antimicrobial production in Streptomyces spp.

The length of Incubation also affects the growth and production of secondary metabolites by an endophytic fungus, *Colletotrichum gloeosporioides* strain LCM 938.01. It determines the physiological state of the fungus which transits from active biomass accumulation to secondary metabolism and ultimately to nutrient depletion and decline phases [29]. This study has shown that both biomass yield and production of antimicrobial metabolites by the fungus increased with incubation time, from 5 to 20 days, with a peak at day 20 (Biomass yield: 229 mg/100 ml; IZD: 16.0 ±0.0 mm against both *E. coli* and *K. pneumoniae*) (Fig. 6A and 6B). This style of growth shows typical fungal growth dynamics, where the transition from log phase to stationary phase is driven by autolysis or nutrient depletion, leading to a decline in biomass yield and the production of antimicrobial metabolites. This supports the findings in *Fusarium solani* and *Penicillium citrinum* [37], [40]. Beyond Day 20, there was a sharp decrease in the growth (Biomass: 147 mg/100ml) and metabolite production (IZD: 10 ± 0.0 mm for *B. subtilis* and *K. pneumoniae*). Beyond that (Day 25), there was a decline in the amount of biomass and antimicrobial metabolites produced. These studies confirm that maximum bioactive metabolite production typically occur during the stationary phase, at peak biomass production. The decline in antimicrobial activity after Day 20 is an indication that secondary metabolites are degraded or inactivated over time [41].

CONCLUSION

The findings of this study have shown that *Colletotrichum gloeosporioides* strain LCM 938.01 grew and produce antimicrobial compounds maximally when cultured on rice medium at the temperature of 25 °C on Day-20 of incubation, with the incorporation of 1 % fructose and glycine as carbon and nitrogen sources respectively into the growth medium. This study shows that both environmental and nutritional factors affect the growth and antimicrobial production in *Colletotrichum gloeosporioides* strain LCM 938.01. However, there is need to structurally elucidate the bioactive compounds for pharmaceutical applications.

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AUTHORS' CONTRIBUTION

OCA conceptualized and drafted the manuscript guidelines. ECC and UIA did the initial drafting of the manuscript. OCE did culture preparation. ESA and OCA were involved in microbiological studies. ODC did the statistical data analysis. BDP and ORC were involved in manuscript editing and reviews. AAA designed and supervised the research.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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REFERENCES

1. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, *109*(7), 2015: 309–318. https://doi.org/10.1179/2047773215y.0000000030
2. Onwudiwe TC, Ughachukwu PO, Unekwe PC, Ogamba JO. Evaluation of antiulcer properties of ethanolic and hot aqueous stem extracts of *Synclisia Scabrida* on experimentally induced ulcer models in albino mice. *Annals of Medical and Health Sciences Research*, 2(2), 2012: 134-139 https://doi.org/10.4103/2141-9248.105660
3. Gunatilaka AA L. Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. *Journal of Natural Products*, *69*(3), 2006: 509–526. https://doi.org/10.1021/np058128n
4. Adonu, C, Ugwueze ME, Ozioko CA. Phytochemical Analyses of Ethanol and Water Extracts of *Millettia aboensis*, *Cuscuta reflexa*, *Daniella oliveri* and *Synclisia scabrida*. International Journal of Pharmacy and Bbiological Sciences, 2013 https://www.academia.edu/69413016/
5. Nnamudi AC, Ebohon, O, Etim, O E. Phytochemical Constituents, Anti nutritional Factors and *In Vitro* Antioxidant Potential of *Synclisia scabrida* Root Extracts. 2020: *32*(3).
6. Khare E, Mishra J, Arora N. Multifaceted Interactions between Endophytes and Plant: Developments and Prospects. *Frontiers in Microbiology*, *9*, 2018, 2732. https://doi.org/10.3389/fmicb.2018.02732
7. Chhipa H, Deshmukh S. Fungal Endophytes: Rising Tools in Sustainable Agriculture Production, 2019: 1–24. https://doi.org/10.1007/978-3-319-76900-4_26-1
8. Malinowski DP, Belesk, DP. Epichloë (formerly Neotyphodium) fungal endophytes increase adaptation of cool-season perennial grasses to environmental stresses. *Acta Agrobotanica*, *72*(2), Article 2. https://doi.org/10.5586/aa.1767
9. Deka D, Jha, D. Optimization of Culture Parameters for Improved Production of Bioactive Metabolite by Endophytic *Geosmithia pallida* (KU693285) Isolated from *Brucea mollis* Wall ex. Kurz, An Endangered Medicinal Plant. *Journal of Pure and Applied Microbiology*, *12*, 2018: 1205–1213. https://doi.org/10.22207/JPAM.12.3.21
10. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews, MMBR*, *67*(4), 2003: 491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003
11. Bhattacharyya P, Jha D. Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a subsurface Aspergillus strain tsf 146. *International Journal of Applied Biology and Pharmaceutical Technology*. 2(4), 2011: 133-143. ISSN 0976-4550.
12. Sharma M, Kulshrestha S. *Colletotrichum gloeosporioides*: An anthracnose causing pathogen of fruits and vegetables. *Biosciences Biotechnology Research Asia*, *12*(2), 2015: 1233–1246.

13. Afanador-Kafuri L, González A, Gañán L, Mejía JF, Cardona N, Alvarez E. Characterization of the *Colletotrichum* species causing Anthracnose in Andean Blackberry in Colombia. Plant Dis 98,2014:1503–1513. https://doi.org/10.1094/PDIS-07-13-0752-RE
14. Lu G, Cannon, PF, Reid, A, Simmons, CM. Diversity and molecular relationships of endophytic Colletotrichum isolates from the Iwokrama Forest Reserve, Guyana. *Mycological Research*, *108*(1), 2004: 53–63. https://doi.org/10.1017/S0953756203008906
15. Muthumary KNJ. Growth studies of *Colletotrichum gloeosporioides* (Penz.). Sacc. - Ataxol producing endophytic fungus from axol producing endophytic fungus from *Plumeria acutifolia. Indian Journal of Science and Technology*, *2*(11), 2009:14–19. https://doi.org/10.17485/ijst/2009/v2i11.10
16. Ozioko CA, Emencheta SC, Eze CC, Agbo MC, Ogbuanya CE., Ibezim EC, Attama, A. A. Antimicrobial Potentials of Secondary Metabolites of Endophytic Fungi Isolated from Synclisia scabrida Miers. *Tropical Journal of Natural Product Research*, *8*(7), Article 7. https://doi.org/10.26538/tjnpr/v8i7.27 2024
17. Devarajan PT. Evidence for the role of phytophagous insects in dispersal of non-grass fungal endophytes..https://www.academia.edu/78226791/Evidence for the role of phytophagous insects in dispersal of non-grass fungal endophytes, 2006.
18. Attia EA, Singh BP, Dashora K, Abdel-Azeem KA. A potential Antimicrobial, Extracellular Enzymes, and Antioxidants resource: Endophytic fungi associated with medicinal plants. Int. J. Biosci., 17(1), 2020): 119-132. DOI: http://dx.doi.org/10.12692/ijb/17.1.119-132
19. Mathan S, Subramanian V, Nagamony S. *Optimization and antimicrobial metabolite production from endophytic fungi Aspergillus terreus KC 582297*, 2013.
20. Sezhian U, Bose P. *Optimization of Culture Conditions for Growth and Production of Bioactive Metabolites by Endophytic Fungus -Aspergillus tamari*, 2013: 469–478. https://doi.org/10.21276/ijpbs.2019.9.2.60
21. Alam B, Lǐ J, Gě Q, Khan M A, Gōng J, Mehmood S, Yuán Y, Gǒng, W. Endophytic Fungi: From Symbiosis to Secondary Metabolite Communications or Vice Versa? *Frontiers in Plant Science*, *12*, 2021. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2021.791033
22. Toppo P, Kagatay LL, Gurung A, Singla P, Chakraborty R, Roy S, Mathur P. Endophytic fungi mediates production of bioactive secondary metabolites via modulation of genes involved in key metabolic pathways and their contribution in different biotechnological sector. *3 Biotechnology*, *13*(6), 2023: 191. https://doi.org/10.1007/s13205-023-03605-z
23. Kumara KW, Rawal R. Influence of Carbon, Nitrogen, Temperature and PH on the Growth and Sporulation of Some Indian Isolates of *Colletotrichum gloeosporioides* causing Anthracnose Disease of Papaya (*Carrica papaya* L). *Tropical Agricultural Research and Extension*, *11*(0), 2010: 7. https://doi.org/10.4038/tare.v11i0.1779
24. Verma S, Lal M, Debnath M. Optimization of process parameters for production of antimicrobial metabolites by an endophytic fungus Aspergillus sp. CPR5 isolated from calotropis Procera root. *Asian Journal of Pharmaceutical and Clinical Research*, *10*, 2017: 225. https://doi.org/10.22159/ajpcr.2017.v10i4.16631
25. Merlin JN, Nimal IVS, Christhuda S, Praveen KP, Agastian P. Optimization of growth and bioactive metabolite production: *Fusarium solani. Asian Journal of Pharmacy and Clinical Research*, 6(3), 2013: 98-103.
26. Gogoi DK, Deka Boruah HP, Saikia R, Bora TC, Optimization of process parameters for improved production of bioactive metabolite by a novel endophytic fungus Fusarium sp. DF2 isolated from *Taxus wallichiana* of North East India. *World Journal of Microbiology and Biotechnology*, 24, 2008:79-87.http://dx.doi.org/10.1051/agro:2008005.
27. Yuniati Y, Monica E, Rollando R. Effect of Variation Conditions Fermentation to Production Biomass of Endophytic Fungi *Athelia rolfsii* Strain orchid. *Journal of Pharmaceutical Sciences & Research*, 10(11), 2018: 2862-2865. ISSN: 0975-1459.

28. Deka D, Jha DK. Optimization of Culture Parameters for Improved Production of Bioactive Metabolite by Endophytic *Geosmithia pallida* (KU693285) Isolated from Brucea mollis Wall ex. Kurz, An Endangered Medicinal Plant. *Journal of Pure and Applied Microbiology*, *12*(3), 2018b: 1205–1213. https://doi.org/10.22207/JPAM.12.3.21
29. Netzker T, Flak M, Krespach MK, Stroe MC, Weber J, Schroeckh V, Brakhageb AA. Microbial interactions trigger the production of antibiotics. *Current Opinion in Microbiology*, *45*, 2018: 23. https://doi.org/10.1016/j.mib.2018.04.002
30. Mattoon ER, Casadevall A, Cordero RJB. Beat the heat: Correlates, compounds, and mechanisms involved in fungal thermo tolerance. *Fungal Biology Reviews*, *36*, 2021: 60–75. https://doi.org/10.1016/j.fbr.2021.03.002
31. Frisvad JC, Andersen B, Thrane U. The use of secondary metabolite profiling in chemotaxonomy of filamentous fungi. *Mycological Research*, *112*(2), 2008: 231–240. https://doi.org/10.1016/j.mycres.2007.08.018
32. Syamsia S, Idhan A., Latifah H., Noerfitryani, N, Akbar A. Alternative medium for the growth of endophytic fungi. *IOP Conference Series: Earth and Environmental Science*, *886*, 2021, 012045. https://doi.org/10.1088/1755-1313.5/886/1/012045
33. AbdelRazek M, Moussa A, Elshanawany M, Singab AN. Optimization of Culture Media for *Halocnemum strobilaceum* Associated Endophytes Bioactivity. *Archives of Pharmaceutical Sciences Ain Shams University*, *0*(0), (2020): 135–144. https://doi.org/10.21608/APS.2020.2004.1044
34. Kalyani P, Botsa S, Laxmi KV. Anil S. Optimization of cultural conditions for biomass and antibacterial metabolite production by *Aspergillus fumigatus* strain MF1. *Hybrid Advances*, *2*, 2023. 100016. https://doi.org/10.1016/j.hybadv.2022.100016
35. Gomaa N, Hashad N, Ibrahim R, Mady M, Abdel-Aziz MS, Moharram FA. Bioactive metabolites and host-specific toxins from endophytic fungi, *Alternaria alternate. Vietnam Journal of Chemistry*, 59(6), 2020: 732–759. **DOI:** 10.1002/vjch.202100099 (doi.org in Bing
36. Adegboye OA, Adeboye OO, Adegboye, AO. High biomass yield observed with sucrose in microbial fermentation studies. *ASRIC Journal on Natural Sciences, 3*(2), 2023: 1–12.
37. Patel P. Influence of Carbon—Nitrogen Supplements and pH on Growth of Sugarcane Stem rot Pathogen *Fusarium Solani* NVS671. *Journal of Fungal Diversity*, *1*(1), 2020: 27–32. https://doi.org/10.14302/issn.2766-869X.jfd-20-3193
38. El-Bondkly AM, El-Gendy MM, Abou-Zied, AM. Biotechnological potential of marine-derived fungi for biomass and metabolite production. *Bioresource Technology, 295*, 2020: 122–131. https://doi.org/10.1016/j.biortech.2019.122131 (doi.org in Bing).
39. Wang Z, Zheng Y, Lai Z, Hu X, Wang L, Wang X, Li, Z, Gao M, Yang Y, Wang Q, Li N. Effect of monosaccharide composition and proportion on the bioactivity of polysaccharides: A review. *International Journal of Biological Macromolecules*, *254*, 2024: 127955. https://doi.org/10.1016/j.ijbiomac.2023.127955
40. Kuma S, Aharwal RP, Jain R, Sandhu SS. Bioactive Molecules of Endophytic Fungi and Their Potential in Anticancer Drug Development. *Current Pharmacology Reports*, *7*(2), 2020: 27–41. https://doi.org/10.1007/s40495-021-00251-y.
41. Sunita B, Deovrat B, Nandita N, Tukaram K, Avinask U. Optimization of Culture Conditions for Production of Bioactive Metabolites by Streptomyces spp. Isolated from Soil. *Advances in Microbiology*,.5(6), 2015. **DOI:** 10.4236/aim.2015.56045.