KAVAKA 61(1): 1-6 (2025) DOI:10.36460/Kavaka/61/1/2025/1-6

Antifungal, Enzymatic, and Plant Growth Promotion Activities of Endophytic Fungi from the Medicinal Plant, *Chromolaena Odorata*

N. Kistu Singh[1]*, M. Shyamkesho Singh[2], and R.R. Pandey[3]

[1]*Department of Botany, Kumbi College, Kumbi - 795 133, Manipur, India.*

[2,3]*Department of Life Sciences (Botany), Manipur University, Canchipur - 795 003, Manipur, India.*

**Corresponding author Email: nkistusingh@gmail.com*

(Submitted on July 13, 2023; Accepted on March 26, 2025)

ABSTRACT

***Chromolaena odorata* is a wild medicinal plant and most of the time neglected as a weed. In traditional medicine, *C. odorata* has been used to treat several ailments by different ethnic communities around the world. Medicinal plants are a storehouse of endophytic fungi which shows various biological activities. In this study, endophytic fungi were isolated from *C. odorata* using different parts. Four endophytic fungi were selected based on preliminary antifungal activity and assessed for their antifungal activity against ten fungal phytopathogens. Maximum inhibition percentage was shown by *Trichoderma* sp. 2 (100%) against *A. alternata*, *A. flavus*, *C. lunata*, *S. oryzae*, and *U. virens,* and least inhibition by *Alternaria* sp. 1 against *A. brassicicola* (33.11%). Three isolates were found to produce protease, lipase, and amylase enzymes. All the isolates produced ammonia, and three isolates produced hydrogen cyanide, but none of the isolates could solubilize phosphate. Strong biocontrol agents are very much needed in the recent trend of chemical-free farming practices.**

Keywords: Medicinal, Endophytic, Antifungal, Phytopathogens, Biocontrol

INTRODUCTION

Chromolaena odorata is a perennial shrub that belongs to the Asteraceae. In several studies, *C. odorata* has been reported to be used in traditional medicine as an anti-inflammatory, antihypertensive, antibacterial, antifungal, antispasmodic, anticancer, antiprotozoal, antioxidant, astringent, diuretic, hepatotropic, and wound healing agent (Sirinthipaporn and Jiraungkoorskhul, 2017; Akinmoladun *et al*., 2007; Phan *et al*., 2001). For a long time, medicinal plants have been exploited to obtain natural bioactive compounds. Over-exploitation leads to ecological imbalance and the loss of biodiversity. The endophytic fungi associated with medicinal plants produced similar or even more potential natural products than the host plants (Kusari and Spiteller, 2012). Endophytic fungi are safe, eco-friendly, economical, require less time to cultivate, and are easily mass-cultivable in artificial culture media. Endophytic fungi refer to those groups of fungi that are found associated with the host plant intercellularly or intracellularly and do not show any negative effect on the normal functioning of the host plant (Arnold *et al*., 2000). After the "Green Revolution", the application of synthetic compounds in the field as fertilizers, fungicides, herbicides, insecticides, etc. has increased enormously. These chemical compounds not only destroys natural soil structure and micro-habitats, but also poses a serious health hazard to animals, especially humans, through the food chain. In the last few years, organic farming has been emphasized as an important alternative to conventional agricultural practices (Reddy, 2010). In organic farming, biocontrol agents play a major role. Till now, there are few effective biocontrol agents on the market, and a search for biocontrol agents of wide application is highly needed. Several researchers have shown the application of endophytic fungi as biocontrol agent (De Silva *et al*., 2019; Latz *et al*., 2018). Endophytic fungi occupying a distinct habitat produce many extracellular enzymes and plant growth-promoting substances (Singh *et al*., 2021; Bhagobaty and Joshi, 2012). These enzymes are used to absorb nutrients from the host cells as well as protect the host plant from the attack of pathogens. Many endophytic fungi were reported to produce ammonia, hydrogen cyanide, and phytohormones, which also help in the uptake and mobilization of essential elements by the host plant. Enzymes of microbial origin are widely used in industries like textiles, food, cosmetics, pharmaceuticals, etc. This study will increase the importance of endophytic fungi in the field of agriculture. This study is the first report of association of endophytic fungi with the host plant *C. odorata*.

MATERIALS AND METHODS

Plant collection: Plant samples were collected from Imphal-East district of Manipur at latitude 24.878347º and longitude 93.950478º. The plant was authenticated and deposited at the Manipur University Museum of Plants (MUMP). The collected plant samples were processed within 24 hrs of collection. Four plant parts, viz., leaf, inflorescence, root, and stem were used for the isolation of endophytic fungi.

Isolation of endophytic fungi: The isolation was conducted according to the protocol given by Hallmann *et al*. (2006). The plant parts were washed in running tap water, then surface sterilized using 70% ethyl alcohol and 4% sodium hypochlorite. The surface sterilized plant segments were cut into 0.5 cm and transferred into petriplates containing solidified potato dextrose agar (PDA) media with added antibiotic streptomycin sulphate and incubated at 28±1º C for 3 to 5 days. The emerged fungal hyphae from the plant segments were pure cultured and proceeded for identification.

Identification of endophytic fungi: The isolates were identified based on morphological characteristics like colony colour, shape, texture, pigmentation, growth rate, and sporulating structures after mounting on lactophenol cotton blue solution (Watanabe, 2010; Barnett and Hunter, 1998). The endophytic fungal isolates were deposited at the NFCCI, Agharkar Institute, Pune.

Antifungal activity: The sporulating isolates were tested for antifungal activity against the plant pathogen *Curvularia lunata*. The culture plug of 9 mm size of the endophyte and the pathogen were inoculated into the petriplate 6 cm apart and incubated at 28±1º C for 10 days. After incubation, the degree of antagonism was given on a scale of class 1 (excellent antagonist), class 2 (strong antagonist), class 3 (good antagonist), class 4 (weak antagonist), and class 5 (non-antagonist) (Bell *et al*., 1982).

Four isolates that show prominent antagonist activity were selected and further evaluated antifungal activity by using the dual culture method against ten fungal plant pathogens: *Curvularia lunata* (ITCC 7170), *Fusarium oxysporum* (ITCC 4998), *Aspergillus niger* (ITCC 5406), *Aspergillus flavus* (ITCC 6972), *Rhizoctonia solani* (ITCC 4576), *Alternaria brassicicola* (ITCC 6193), *Colletotrichum capsici* (ITCC 6078), *Ustilaginoidea virens* (ITCC 7046), *Alternaria alternata* (ITCC 6778), and *Sclerotium oryzae* (ITCC 6972). After seven days of incubation, inhibition percentage (I%) was calculated by using the formula, I% = [(r1-r2)/r1] × 100; r1 = mycelial growth of the pathogen on the control plate, r2 = mycelial radial growth of the pathogen on the dual culture plate (Yu *et al*., 2018).

Qualitative enzyme activities: The production of five extracellular enzymes were assessed by the digestion of dissolved substrates by the isolates (Sharma *et al*., 2020; Rajput *et al*., 2016).

Protease activity: The isolates were inoculated on glucose yeast peptone agar (GYPA) media along with skim milk for five days and observed for the appearance of a clear zone around the colony.

Lipase activity: The endophytes were inoculated with the peptone agar (PA) medium containing Tween 20. The appearance of a clear zone after incubation shows lipase activity.

Amylase activity: The isolates were inoculated on GYPA media with 1% soluble starch and incubated. The iodine solution (1%) was poured on each plate and left for 15 secs and observed for the appearance of a clear zone around the colony.

Cellulase activity: The endophytic fungi were inoculated on GYPA media with carboxy methyl cellulose (CMC). After incubation, 1% Congo red solution was flooded on each plate for 20 mins and then washed with NaCl solution for 15 mins. The appearance of a light-yellow area around the fungal colonies indicates the release of the cellulase enzyme.

Laccase activity: The fungal endophytes were inoculated on GYPA media supplemented with 1-naphthol. The colour change of the medium into blue/purple shows laccase activity.

Plant growth promotion activities: The isolates were tested for phosphate solubilization, ammonia production, and hydrogen cyanide production to assess plant growth promotion abilities.

Phosphate (PO_4) solubilization: The isolates were inoculated on petriplates with Pikovskaya's agar medium with added calcium phosphate. The petriplates were incubated at 28±1°C for seven days. The appearance of a clear zone around the colony indicates the solubilization of phosphate (Ripa *et al*., 2019).

Ammonia (NH_4) production: The endophytes were inoculated in 10 ml peptone water and incubated for 72 hrs at 28±1°C. After incubation, 0.5 ml of Nessler's reagent was added. The development of a brown to yellow colour shows the production of ammonia (Mahfooz *et al*., 2017).

Hydrogen cyanide (HCN) production: The Bennett agar media was used for HCN activity. The fungal isolates were inoculated in Bennett agar test tubes. The filter paper Whatman no. 1 flooded with a solution of picric acid and sodium carbonate were air dried and attached inside the test tube wall and incubated at 28±1°C for ten days. The colour change of the filter paper from light yellow to brown or red gives positive result for HCN activity (Passari *et al*., 2016).

RESULTS

Isolation: The endophytic fungi were isolated from all the parts of *Chromolaena odorata* (L.) R. M. King and H. Rob. (MUMP accession no. – 001368) (**Figure 1**).

Figure 1: *Chromolaena odorata.* a, Plant; b, Isolation of endophytic fungi

Identification: A total of fifteen endophytic fungi were identified and three isolates were found to be sterile. The isolates were grouped under nine genera and nine families (**Table 1**, **Figure 2**).

Table 1: Identification of endophytic fungi from *Chromolaena odorata*.

Isolate code	Endophytic fungi	NFCCI accession	Family
COR3B3	*Alternaria* sp. 1	*	Pleosporaceae
COS5C5	*Arthrinium* sp. 2	NFCCI 5416	Apiosporaceae
COS5G5	*Aspergillus* sp. 1	NFCCI 5418	Aspergillaceae
COS2B3	*Aspergillus* sp. 2	NFCCI 5420	Aspergillaceae
COS3B1	*Aspergillus* sp. 3	NFCCI 5422	Aspergillaceae
COL1C2	*Aspergillus* sp. 4	NFCCI 5414	Aspergillaceae
COR1C1	*Chaetomium* sp.	NFCCI 5423	Chaetomiaceae
COL3A3	*Cladosporium* sp.	*	Cladosporiaceae
COR4A4	*Fusarium* sp. 1	NFCCI 5421	Nectriaceae
COF3A3	*Fusarium* sp. 2	*	Nectriaceae
COF2A2	*Mucor* sp.	NFCCI 5412	Mucoraceae
COS1A1	*Penicillium* sp. 1	NFCCI 5417	Aspergillaceae
COR1A1	*Penicillium* sp. 2	NFCCI 5413	Aspergillaceae
COS5A5	*Trichoderma* sp. 1	NFCCI 5415	Hypocreaceae
COS2A2	*Trichoderma* sp. 2	NFCCI 5419	Hypocreaceae
COL4E4	Sterile morphotype 1	**	
COF1A1	Sterile morphotype 2	**	
COL2E2	Sterile morphotype 3	**	

*, Applied for accession number; **, No accession number for sterile form

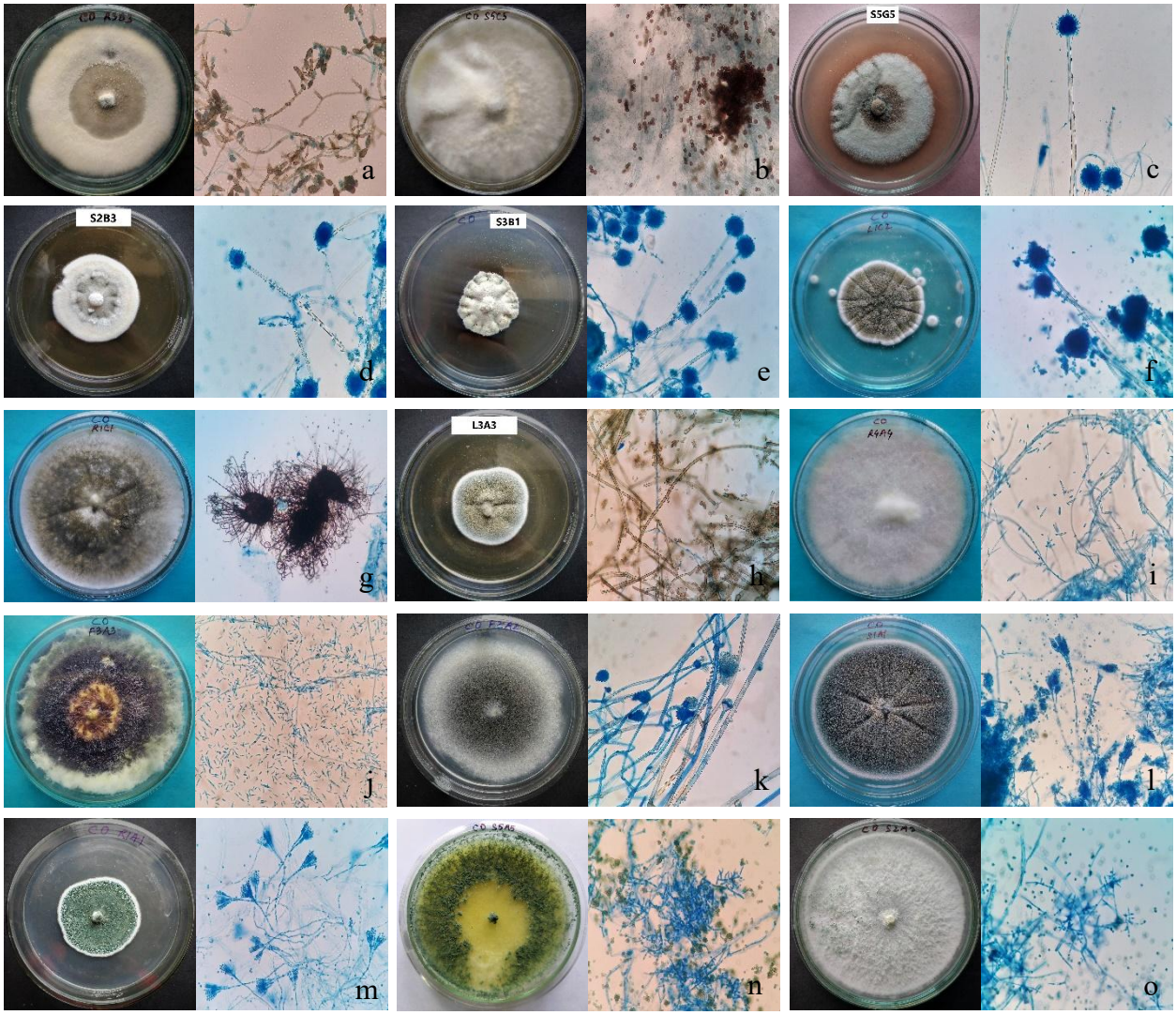

Figure 2: Morphological photographs of a, *Alternaria* sp.; b, *Arthrinium* sp.; c, *Aspergillus* sp. 1; d, *Aspergillus* sp. 2; e, *Aspergillus* sp. 3; f, *Aspergillus* sp. 4; g, *Chaetomium* sp.; h, *Cladosporium* sp.; i, *Fusarium* sp. 1; j, *Fusarium* sp. 2; k, *Mucor* sp.; l, *Penicillium* sp. 1; m, *Penicillium* sp. 2; n, *Trichoderma* sp. 1; o, *Trichoderma* sp. 2.

Antifungal activity: Preliminary antifungal activity shows that out of the fifteen isolates, two isolates were class 1, five isolates class 2, one isolate class 3, seven isolates class 4, and none of the isolate was found to be class 5 (**Figure 3**).

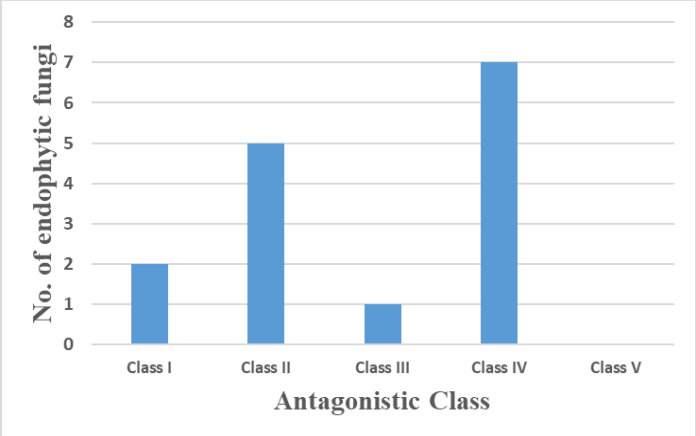

Figure 3: Antagonistic classes of endophytic fungi against *Curvularia lunata*.

Further evaluation of antagonistic inhibition percentage (I%) of the four isolates has shown that *Trichoderma* sp.2 was the most potent with 100% inhibition against *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Sclerotium oryzae*, and *Ustilaginoidea virens*; the least inhibition was shown by *Alternaria* sp.1 against *Alternaria brassicicola* (**Table 2**).

Qualitative enzyme activity and plant growth promotion abilities: All the four isolates produce protease, amylase, lipase, and ammonia; cellulase was produce by *Fusarium* sp.2, *Trichoderma* sp.1, and *Trichoderma* sp.2; *Alternaria* sp., laccase was produced by *Trichoderma* sp.1, and *Trichoderma* sp.2; and none of the isolates could solubilize phosphate (**Table 3**).

Table 2: Inhibition percentage (I%) of four isolates of endophytic fungi against phytopathogens.

Pathogens	Inhibition percentage (I%) shown by endophytic fungi			
	***Alternaria* sp. 1**	***Fusarium* sp. 2**	***Trichoderma* sp. 1**	***Trichoderma* sp. 2**
Alternaria alternata	36.72±0.06	34.56±0.06	79.91±0.03	100.00±0.00
Alternaria brassicicola	33.11±0.05	63.13±0.06	83.00±0.00	84.55±0.06
Aspergillus flavus	72.12±0.06	66.34±0.03	100.00±0.00	100.00±0.00
Aspergillus niger	55.18±0.08	58.14±0.00	69.97±0.06	97.04±0.05
Colletotrichum capsici	63.89±0.03	56.46±0.08	81.72±0.10	87.07±0.06
Curvularia lunata	59.35±0.03	68.88±0.06	100.00±0.00	100.00±0.00
Fusarium oxysporum	30.23±0.00	42.65±0.05	79.92±0.06	81.37±0.06
Rhizoctonia solani	43.37±0.05	34.80±0.00	78.58±0.06	82.19±0.03
Sclerotium oryzae	46.12±0.06	52.81±0.06	91.58±0.03	100.00±0.00
Ustilaginoidea virens	63.02±0.10	75.61±0.06	100.00±0.00	100.00±0.00

Data are mean ± standard deviation (SD)

Table 3: Qualitative analysis of enzyme production and plant growth promotion abilities.

Endophytic fungi	Enzyme production					Growth promotion		
	Protease	**Lipase**	**Amylase**	**Cellulase**	**Laccase**	**NH_4**	**PO_4**	**HCN**
Alternaria sp.	+	+	+	-	-	+	-	+
Fusarium sp. 2	+	+	+	+	-	+	-	-
Trichoderma sp. 1	+	+	+	+	-	+	-	+
Trichoderma sp. 2	+	+	+	+	+	+	-	+

+, indicates presence; -, indicates absence

DISCUSSION

Medicinal plants with ethnobotanical importance are considered to be associated with diverse endophytic fungi with vast biological applications. The scope of endophytic research has broadened due to the shift from conventional agriculture to organic agriculture. The major drawback of organic farming is the limited availability of biocontrol agents with wide applicability. Till now, few fungal biocontrol agents are known, such as *Trichoderma* spp. Surface sterilization is the most crucial step in isolating endophytic fungi. In the study, endophytic fungi were found to be associated with all parts of *Chromolaena odorata*. The antagonistic activity revealed that all the isolates possessed antifungal properties, and the most potent was found to be *Trichoderma* sp. 2. The isolates *Fusarium* sp. 2, *Trichoderma* sp. 1, and *Trichoderma* sp. 2 produced protease, lipase, and amylase. Enzymes of microbial origin are industrially important because they are cheaper, reliable, less time-consuming, and easily controllable. All the isolates produce ammonia and supply nitrogen to the host plant, as nitrogen is needed in the synthesis of nucleic acids, chlorophylls, proteins, etc. The isolate, *Trichoderma* sp. 2 produce hydrogen cyanide, which is a volatile compound that protects host plants from pathogens, herbivores, and insects. Further studies are required in the quantification of enzyme production, extraction of secondary metabolites, and other biological activities.

REFERENCES

Akinmoladun, A.C., Ibukun, E.O., Dan-Ologe, I.A. 2007. Phytochemical constituents and antioxidant properties of extracts from the

leaves of *Chromolaena odorata*. *Scientific Research and Essay*, **2(6)**:191-194.

Arnold, A.E., Maynard, Z., Gilbert, G.S., *et al.*, 2000. Are tropical fungal endophytes hyperdiverse?. *Ecology letters*, **3(4)**:267-274.

Barnett, H.L. and Hunter, B.B. 1998. Illustrated genera of imperfect fungi. The American phytopathological society. *US Department of Agriculture, Agricultural Research Service, Washington State University, Pullman. APS Press. USA. St. Paul, Minnesota USA. 218p*.

Bell, D.K., Wells, H.D., Markham, C.R. 1982. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*, **72(4)**: 379-382.

Bhagobaty, R.K. and Joshi, S.R. 2012. Enzymatic activity of fungi endophytic on five medicinal plant species of the pristine sacred forests of Meghalaya, India. *Biotechnology and bioprocess engineering*, **17**:33-40.

De Silva, N.I., Brooks, S., Lumyong, S., *et al.*, 2019. Use of endophytes as biocontrol agents. *Fungal Biology Reviews*, **33(2)**:133-148.

Hallmann, J., Berg, G., Schulz, B. 2006. Isolation procedures for endophytic microorganisms. In *Microbial root endophytes* (pp.299-319), Springer. Berlin, Heidelberg.

Kusari, S., and Spiteller, M. 2012. Metabolomics of endophytic fungi producing associated plant secondary metabolites: progress, challenges and opportunities. *Metabolomics,* 241-266.

Latz, M.A., Jensen, B., Collinge, D.B., *et al.*, 2018. Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology and Diversity*, **11(5-6)**:555-567.

Mahfooz, M., Dwedi, S., Bhatt, A., *et al.*, 2017. Evaluation of Antifungal and Enzymatic Potential of Endophytic Fungi Isolated from *Cupressus torulosa* D. Don. *International Journal of Current Microbiology and Applied Sciences,* **6(7)**:4084-4100.

Passari, A. K., Mishra, V. K., Leo, V. V., Gupta, V. K., and Singh, B. P. (2016). Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. *Microbiological research*, *193*, 57-73.

Phan, T. T., Wang, L., See, P., Grayer, R. J., Chan, S. Y., and Lee, S. T. (2001). Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. *Biological and Pharmaceutical Bulletin*, *24*(12), 1373-1379.

Rajput, K., Chanyal, S., and Agrawal, P. K. (2016). Optimization of protease production by endophytic fungus, *Alternaria alternata* isolated from gymnosperm tree-*Cupressus torulosa* D Don. World J. Pharm. Pharmaceut. Sci, 5, 1034-1054.

Reddy, B. S. (2010). Organic farming: status, issues and prospects–a review. *Agricultural Economics Research Review*, *23*(347-2016-16927), 343-358.

Ripa, F. A., Cao, W. D., Tong, S., and Sun, J. G. (2019). Assessment of plant growth promoting and abiotic stress tolerance properties of wheat endophytic fungi. BioMed Research International, 2019.

Sharma, Sneh, Varsha Rani, Raj Saini, and Madan L. Verma. "Bioprospecting and biotechnological applications of microbial endophytes." *Microbial Technology for Health and Environment* (2020): 191-228.

Singh, N., Singh, A., and Dahiya, P. (2021). Plant growth-promoting endophytic fungi from different habitats and their potential applications in agriculture. *Recent Trends in Mycological Research: Volume 1: Agricultural and Medical Perspective*, 69-87.

Sirinthipaporn, A., and Jiraungkoorskul, W. (2017). Wound healing property review of siam weed, Chromolaena odorata. *Pharmacognosy reviews*, *11*(21), 35.

Watanabe, T. (2010). *Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species*. CRC press.

Yu, J., Wu, Y., He, Z., Li, M., Zhu, K., and Gao, B. (2018). Diversity and antifungal activity of endophytic fungi associated with *Camellia oleifera*. *Mycobiology*, *46*(2), 85-91.