Thermotolerant Filamentous Fungi in the Rhizosphere of Banana Trees in the Agroecological Zone of Divo, Southwestern Côte d'Ivoire

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ABSTRACT

Despite the negative impact of climate change on Ivorian agriculture, the department of Divo accounts for 18% of national plantain production. This resilience can be partly attributed to the activity of filamentous fungi in the rhizosphere. The aim of this study was to identify thermotolerant filamentous fungi associated with the rhizospheric soils of banana trees in this department. Soil samples were taken from four banana plantations, followed by microbiological analyses to isolate the fungi. A selection process identified fungal isolates capable of withstanding a temperature of 55°C, confirming their thermotolerance. The results revealed a fungal diversity comprising the genera *Aspergillus*, *Absidia*, *Paecilomyces*, *Penicillium*, *Scedosporium*, *Mucor*, *Trichoderma*, *Beauveria*, and *Alternaria*. With the exception of *Alternaria*, all these genera presented isolates with thermotolerance. The presence of these microorganisms in the rhizosphere of banana trees is a major agronomic asset. By facilitating the decomposition of organic matter and protecting plants against pathogens, they help improve crop productivity in the face of environmental stress. This research has therefore identified thermotolerant fungi that are likely to play a key role in the resistance of banana trees to global warming.

Keywords: banana trees, thermotolerant, filamentous fungi, rhizospheric soils

1. INTRODUCTION

Climate change poses a major challenge for agriculture in tropical Africa, particularly in Côte d'Ivoire, where it manifests itself in lower rainfall and higher temperatures (Affoué et al., 2019). These climatic anomalies have significant repercussions on agricultural production, particularly on plantains, an essential crop that requires a lot of water and is vulnerable to extreme temperatures (Chanzy et al., 2015). In Côte d'Ivoire, this plant plays a crucial role both in terms of food and the economy. It ranks as the country's third most important food crop, after yams and cassava (Traoré et al., 2009). The plantain industry is an important source of income for farmers, with an annual production of around 2.3 million tons of bananas. In addition, it contributes between 5 and 8% of the national agricultural GDP and generates around 22,000 jobs (CIRAD, 2023; FAO, 2023). Plantain cultivation is mainly concentrated in the eastern, central-western, southwestern, and western parts of the country (Thiémélé, 2017).

Despite the challenges posed by climate change, plantain production in the department of Divo, located in the Lôh-Djiboua region in the southwest, remains significant, accounting for approximately 18% of national production (FAO, 2023). This resilience to changing climatic conditions can be attributed to several biotic and abiotic factors, among which filamentous fungi play an essential role in plant health and productivity. Abundant in the rhizosphere, these fungi have a remarkable ability to adapt to extreme conditions, particularly high temperatures (Jaegers, 2024). Under such circumstances, some fungi that are generally mesophilic can evolve to become thermotolerant. These thermotolerant fungi are particularly important because they actively participate in the decomposition of organic matter, which enriches the soil with nutrients essential for plant growth (Van-Der-Heijden & Hartmann, 2016). In addition, by releasing these nutrients, they improve the availability of nutrients for plantain, thereby promoting its development. They can also protect banana trees against several pathogens by establishing beneficial interactions with the roots (Ridout & Newcombe, 2016).

Therefore, knowledge of the thermotolerant fungi present in the rhizosphere of banana plants is crucial to understanding their influence on the development, productivity, and health of this plant, particularly in the context of climate change. The overall objective of this study is to identify thermotolerant filamentous fungi in the rhizosphere of banana plants in the department of Divo.

2. Materials and methods

2.1. Study area

The rhizosphere soil samples from plantains used to isolate thermotolerant fungi were collected from four (04) plantain fields (Table I) in southwestern Côte d'Ivoire in the department of Divo. This department, located in the Lôh-Djiboua region at geographical coordinates 5°50’00” North and 5°22’00” West, is a major plantain-producing area. The soil is ferralitic and the climate is equatorial (hot and rainy) with dense forest vegetation (Ourega & Gbocho, 2021).

Table I: Geographic coordinates of banana plantations

Fields	Geographical coordinates
Field 1	5°44’07” North and 4°51’20” West
Field 2	5°47’24” North and 4°54’00” West
Field 3	5°51’00” North and 5°20’00” West
Field 4	5°45’00” North and 5°46’00” West

2.2. Rhizosphere soil sampling

Rhizosphere soil sampling took place during July 2024 in four (04) banana plantations in the department of Divo. This operation was carried out using the point and plot sampling method (Vincent, 2007). Three plots at least 50 m apart were defined in each banana plantation. Samples were taken within a radius of 0.5 m around the base of each banana tree. The soil surface was cleared of plant and animal debris using a hoe to a depth of 15 to 20 cm. Fifty grams of soil adhering to the roots of the plantain trees included in each defined plot were collected using an auger (Meddich et al., 2017). A composite sample per plot was produced by mixing and homogenizing all the samples from a given plot. A total of 12 samples of approximately 500 g of rhizosphere soil were collected, packaged in sterile bags, and sent to the laboratory for further analysis.

2.3. Isolation of rhizosphere fungi

A quantity of 5 to 10 mg of each previously dried soil sample was weighed and placed in a Petri dish. Approximately 20 ml of Sabouraud medium with chloramphenicol, prepared and maintained at a supercooled temperature of 45 to 50 °C, was added. The weighed soil was immediately dispersed in the culture medium by gently shaking the Petri dish (Warcup, 1950).

Three tests were carried out per soil sample. Incubation took place at 30°C for seven days, after which the morphologically different colonies observed on the Petri dishes were collected and cultured individually under the same conditions.

2.4. Identification and selection of thermophilic fungi

The fungal isolates were characterized based on morphological observations. Macroscopic characteristics, namely the color, shape, and appearance of the mycelium on Sabouraud agar with chloramphenicol, were observed. In addition, microscopic identification based on the mode of hyphal partitioning, the shape of conidia, and the presence of phialides and chlamydospores was performed on 7-day-old colonies using the Scotch tape method at 40x magnification (Carlier et al., 2002). Morphological identification of fungi does not allow species to be clearly distinguished, thus limiting identification to genera. To accurately identify species, it is therefore essential to use molecular methods.

The selection of thermotolerant fungi was carried out by re-incubating the identified molds in an oven at a temperature of 55°C for a period of 3 to 7 days (Verscheure et al., 2002). At this high temperature, only thermotolerant molds will continue to grow. Three replicates were performed for each reincubated isolate. To evaluate the thermotolerant capacity of these fungi, their growth rate (V) at 55°C was determined using the following formula: V = Average diameter (mm)/Time (days). This rate was then compared to that observed at 30°C.

2.5. Fungal load in rhizospheric soils (CFU/g of soil)

The microbial load of soil samples was calculated using the following equation (Chabasse, 2024):

$$N(UFC/g) = \frac{\sum Ci}{0{,}01N'}$$

-N (CFU/g): Number of germs per gram of soil;

-N': Number of plates used for counting;

-∑Ci: Sum of colonies counted on all Petri dishes used; -0.01: Gram of soil used for each Petri dish.

2.6. Statistical analysis

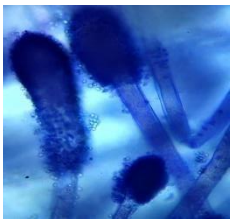
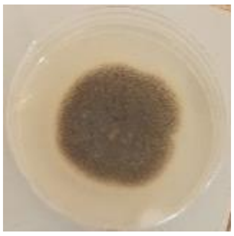
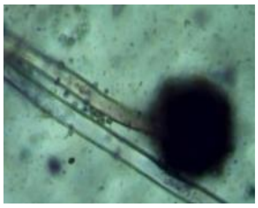
The collected data were analyzed using R software version 4.4.1 (RStudio). Due to non-compliance with the conditions of normality and homogeneity of variances, soil fungal loads were compared using a Generalized Linear Model (GLM) with a Gaussian link function. The significance of the effects was assessed by a deviance analysis coupled with the χ^2 (chi-square) test. In addition, the radial growth rates of fungi at 30°C and 55°C were compared using Student's t-test. For all analyses, the significance threshold was set at $p<0.05$.

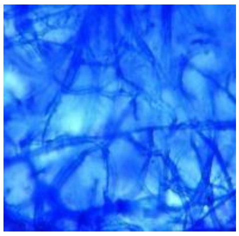
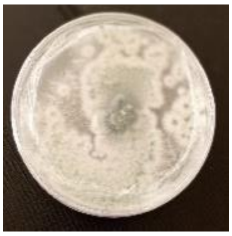
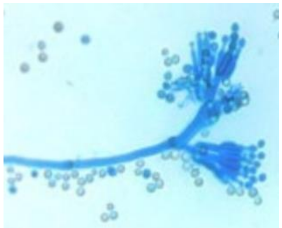
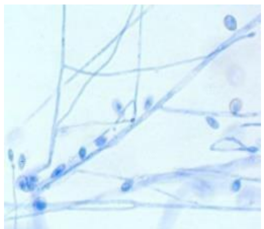
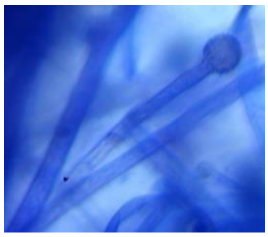
3. RESULTS

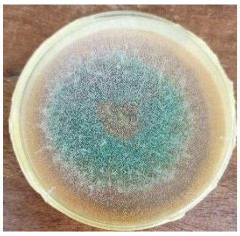
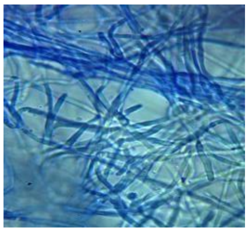
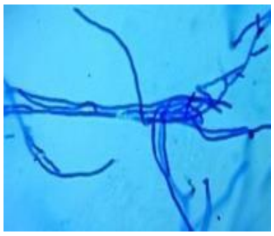
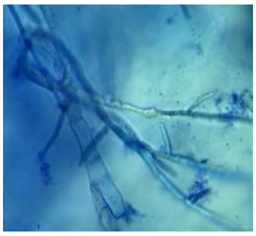
3.1. Fungi isolated from the rhizosphere of banana trees

Microbiological analysis of rhizosphere soils sampled from banana plantations in the department of Divo yielded 34 isolates. Using identification keys incorporating macroscopic and microscopic characteristics, these isolates were classified into nine genera of fungi, including *Aspergillus* (present in two morphotypes), *Absidia*, *Paecilomyces*, *Penicillium*, *Scedosporium*, *Mucor*, *Trichoderma*, *Beauveria*, and *Alternaria*. The macroscopic and microscopic characteristics of these fungal genera are presented in Table II.

Table II: Fungi isolated from the rhizosphere of banana trees in the department of Divo

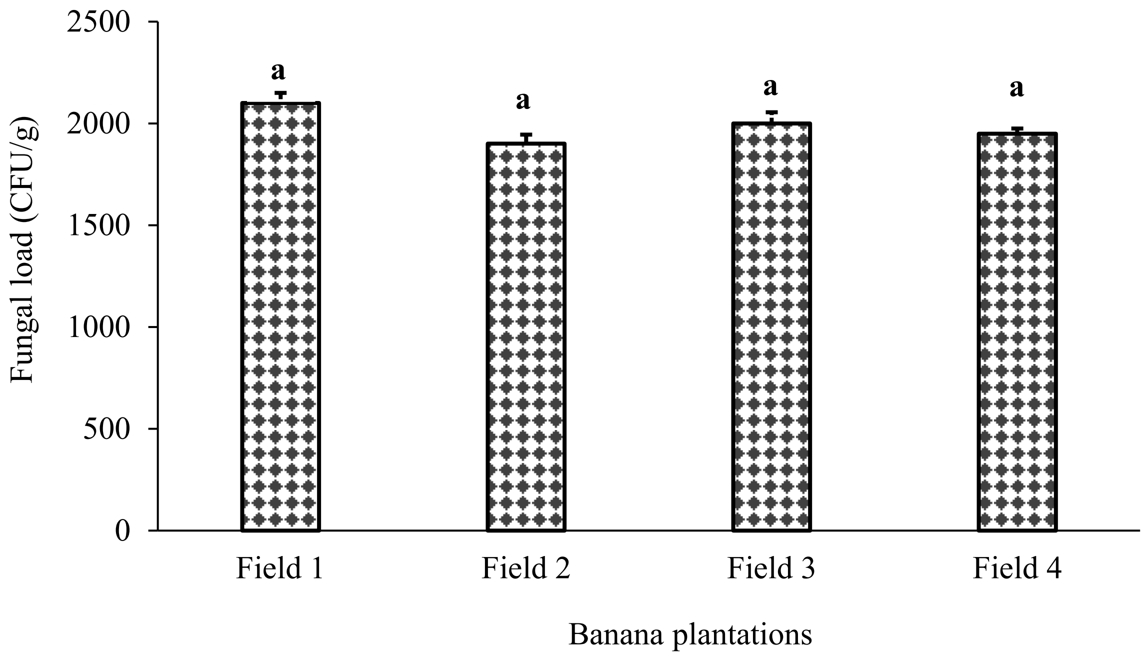
Isolated codes	Genera	Macroscopic aspects	Microscopic aspects
D1, D2, D3,	*Aspergillus*		
D4, D5			

D6, D7, D8	*Absidia*		
D9, D10, D11 , D12, D13, D14	*Paecilomyces*		
D15, D16, D17, D18, D19, D20	*Pénicillium*		
D21, D22, D23	*Scedosporium*		
D24, D25, D26, D27, D28	*Mucor*		

D29, D30	*Trichoderma*		
D31, D32	*Beauveria*		
D33, D34	*Alternaria*		

3.2. Fungal load in the banana plantations investigated (CFU/g of soil)

The loads of fungi isolated from the rhizosphere soils of the four banana plantations in the department of Divo are shown in the figure below. All banana plantations had statistically identical loads.



The bands assigned the same letter are identical according to the χ^2 test at a threshold of a = 5%.

Figure 1: Fungal load in the rhizosphere of the banana plantations investigated

3.3. Thermotolerant fungal flora isolated from the rhizosphere

The 34 mold isolates, initially cultured at 30°C, were re-incubated at 55°C for 3 to 7 days in order to select thermotolerant molds. Of these isolates, only 16 continued to grow at this high temperature (Table III). They were therefore identified as thermotolerants. These were isolates of *Aspergillus* (D4 and D5), *Absidia* (D7), *Paecilomyces* (D10, D11, and D12), *Penicillium* (D16, D17, and D18), *Scedosporium* (D22), *Mucor* (D25 and D26), *Trichoderma* (D29 and D30), and *Beauveria* (D31 and D32). The isolates of *Aspergillus*, *Paecilomyces*, *Penicillium*, and *Trichoderma* showed consistent growth rates regardless of incubation temperature. In contrast, the isolates of *Scedosporium*, *Absidia*, and *Mucor* showed higher growth rates at 30°C than at 55°C, where growth rates were significantly reduced. *Beauveria* isolates showed maximum growth at 55°C, with low growth rates at 30°C.

Table III: Growth rates of rhizospheric fungi as a function of incubation temperature

Genera	Isolates	Growth rate (mm/day) at 30°C	Growth rate (mm/day) at 55°C
Aspergillus	D4	11.43 ± 0.55[a]	10.14 ± 0.35[a]
	D5	10.86 ± 0.75[a]	10.43 ± 0.87[a]
Absidia	D7	7.86 ± 0.15[a]	5 ± 0.65[b]
Paecilomyces	D10	11.43 ± 0.15[a]	10 ± 0.05[a]
	D11	10.28 ± 0.25[a]	9.86 ± 0.02[a]
	D12	10.14 ± 0.45[a]	10.43 ± 0.65[a]
Penicillium	D16	10.71 ± 0.00[a]	10.28 ± 0.15[a]
	D17	11.14 ± 0.95[a]	10.85 ± 0.10[a]
	D18	11.14 ± 0.01[a]	11.28 ± 0.25[a]
Scedosporium	D22	11.43 ± 0.00[a]	7.14 ± 0.71[b]
Mucor	D25	20 ± 0.00[a]	12.5 ± 0.75[b]
	D26	20 ± 0.00[a]	12 ± 0.70[b]
Trichoderma	D29	18.75 ± 0.10[a]	17.5 ± 0.01[a]
	D30	18.50 ± 0.23[a]	17 ± 0.00[a]
Beauveria	D31	7.14 ± 0.17[b]	10 ± 0.03[a]
	D32	7.28 ± 0.20[b]	10.28 ± 0.38[a]

Values assigned the same letter are identical according to the χ^2 test at a threshold of a = 5%. This is a pairwise comparison of growth rates at 30°C and 55°C.

4. DISCUSSION

The objective of this study was to identify thermotolerant fungi associated with the rhizosphere of plantain trees in the Divo area. Microbiological analysis of soil samples taken from four banana plantations revealed a diversity of fungal agents present in these plantations. The fungal loads of the soil samples were statistically identical, suggesting that this homogeneity is linked to similar cultivation practices observed by producers, as indicated by Zain et al. (2014). Indeed, these authors emphasized in their work that cultivation practices strongly influence the microbial ecosystem of soils.

Among the fungi isolated from the rhizosphere soils of banana plants, eight (08) genera presented thermophilic isolates. These are the genera *Aspergillus*, *Absidia*, *Paecilomyces*, *Penicillium*, *Scedosporium*, *Mucor*, *Trichoderma*, and *Beauveria*. Numerous studies confirm the thermotolerant nature of certain species belonging to these genera (Mulaw et al., 2013; Bilgo et al., 2025). The ability of these microorganisms to grow at high temperatures is particularly advantageous in the current context of global warming, which is destabilizing the banana tree ecosystem. These microorganisms are characterized by their rapid growth and high saprophytic competitiveness at extreme temperatures (Bonduelle et al., 2025). This allows them to thrive in conditions where other organisms cannot survive, thereby strengthening their role in the ecosystem. The species *Aspergillus*, *Scedosporium*, *Mucor*, and *Absidia* spend most of their life cycle as saprophytes in the soil (Chabasse, 2024). In nature, these species overwinter in the form of vegetative hyphae or thanks to resistant structures called sclerotia. Their presence in banana plantations is crucial, as these saprophytic microorganisms facilitate the degradation of organic matter at high temperatures, as stated by Ridout & Newcombe (2016). They thus contribute to the biological balance of banana plantation soils, transforming organic matter into mineral matter and renewing humus. As for the species *Paecilomyces*, *Penicillium*, *Trichoderma*, and *Beauveria*, in addition to their saprophytic capacity, they are known for their powerful antagonistic effect against plant pathogens. According to the work of Jaegers (2024), these antagonists are able to control the growth of other fungi, particularly pathogens, through the production of antifungal substances. For example, to limit the development of a pathogen, *Beauveria* species generally produce secondary metabolites such as beauvericin, bassianolide, cyclosporine, beauverolide, isarolid, and oosporein, which can accelerate the fungal infection process and weaken the host's immune system (Yin et al., 2022). *Paecilomyces*, *Penicillium*, and *Trichoderma* species also inhibit pathogen development by producing potent secondary metabolites such as flavonoids, terpenoids, alkaloids, and ketones (Nawaz et al. 2018). These fungi can also act as natural antibacterials and nematicides, while stimulating plant resistance (Van-Der-Heijden & Hartmann, 2016).

5. CONCLUSION

This study revealed the diversity of rhizosphere fungi in plantains in the Divo department of Côte d'Ivoire, with eight genera identified as thermotolerant. These are the genera *Aspergillus*, *Absidia*, *Paecilomyces*, *Penicillium*, *Scedosporium*, *Mucor*, *Trichoderma*, and *Beauveria*. These microorganisms play a crucial role, not only in decomposing organic matter, thereby enriching the soil with nutrients, but also in protecting banana trees from various pathogens. Their ability to adapt to high temperatures makes them valuable allies for the resilience of plantain cultivation, especially in the context of global warming. Furthermore, their ability to promote plant health and stimulate growth through beneficial interactions highlights the importance of understanding and preserving these organisms in agricultural practices. To ensure the sustainability of plantain production in Côte d'Ivoire, it is therefore essential to integrate the management of thermotolerant fungi into agricultural strategies. This could include adapted cultural practices that promote their development and effectiveness, thereby contributing to food security and the economic sustainability of farmers in the region.

6. REFERENCES

Affoué, B.Y., Kouakou, L.K., Koffi, E.K., Bi-Tié, A.G., & Amadou, T.G. (2019). Assessment of the potential impacts of climate change on river flows in the Lobo River, central-western Côte d'Ivoire. *Afrique Science*, 15(4), 330-342.

Bilgo, E., Lamy, D. L., Sawadogo, W. A., & Diabate, A. (2025). Optimization of the isolation of entomopathogenic fungi *Metarhizium* spp. associated with the rhizosphere and insects in western Burkina Faso as part of the fight against malaria. *Sciences Naturelles et Appliquées*, 43(2(1), 177-197.

Bonduelle, M., Desrochers-Noiseux, F., Durand, A.A., Barnabé, S., & Constant, P. (2025). The establishment of microbial communities to promote the growth of Pleurotus ostreatus through a top-down approach is hampered by the dominance of antagonistic interactions. *Applied and Environmental Microbiology*, 91(9), e00898-25.

Carlier, J., De-Waele, D., & Escalant, J.V. (2002). Global Evaluation of *Musa* Germplasm for Resistance to Fusarium Wilt, Mycosphaerella Leaf Spot Diseases and Nematodes. Technical Guides No. 7 of the International Network for the Improvement of Banana and Plantain, Montpellier (France), 62p.

Chabasse, D. (2024). Role of the laboratory in the diagnosis of onychomycosis: methods and interpretation. *Revue Francophone des Laboratoires*, 2024(566), 34-46.

Chanzy, A., Martin, G., Colbach, N., Gosme M., Launay, M., Loyce, C., & Novak, S. (2015). Adaptation of crops and cropping systems to climate change and new uses. Technical mission report from the French National Institute for Agricultural Research, Paris (France), 5p.

CIRAD (2023). Banana statistics compendium. 2023 annual report, Abidjan (Ivory Coast), 40p.

FAO (2023). Banana Market Review – Preliminary results 2023. Annual report on the banana sector, Italy (Rome), 20p.

Jaegers, F.C. (2024). Rooting for Resilience: Unveiling Absorptive Fine Root Traits and Fungal Dynamics in Young Temperate Trees Under High and Low Growing Season Water Availability. Doctoral thesis, UFR de la Biologie, Université du Québec à Montréal (Canada), 216 p.

Meddich, A, Ait El Mokhtar, M, Wahbi, S, Boumezzough, A. (2017). Assessment of mycorrhizal potential in relation to the physicochemical parameters of palm grove soils in Morocco (Marrakesh and Tafilalet). *Cah. Agricole*, 26, 45012.

Mulaw, T.B., Druzhinina, I.S., Kubicek, C.P., & Atanasova, L. (2013). Novel endophytic *Trichoderma* spp. isolated from healthy Coffea arabica roots are capable of controlling coffee tracheomycosis. *Diversity*, 5(4), 750-766.

Nawaz, K. Shahid, A.A, Bengyella, L., Subhani, M.N, Ali, M., Anwar, W., Iftikhara, S. & Ali, S.W. (2018). Diversity of Trichoderma species in chili rhizosphere that promote vigor and antagonism against virulent *Phytophthora capsici*. *Scientia Horticulturae*, 239, 242-252.

Ourega, D.D., & Gbocho, O.D. (2021). Resilience of households in the city of DIVO (Southwest Forest, IVORY COAST) in the face of increasing household waste. *Espace Géographique et Société Marocaine*, 1(54), 111-125.

Ridout, M., & Newcombe, G. (2016). Disease suppression in winter wheat from novel symbiosis with forest fungi. *Fungal Ecology*, 20, 40-48.

Thiémélé, D., Traoré, S., Aby, N., Gnonhouri, P., Yao, N., Kobenan, K., Konan, E., Adiko, A., & Zakra, N. (2017). Diversity and participatory selection of productive local plantain varieties in Côte d'Ivoire. *Journal of Applied Biosciences*, 114, 11324-11335.

Traore, S., Kobenan, K., Kouassi, S., & Gnonhouri, G.P. (2009). Plantain cultivation systems and methods of controlling pests and diseases in smallholder farming in Côte d'Ivoire. *Journal of Applied Biosciences*, 19, 1094-1101.

Van-Der-Heijden, M.G., & Hartmann, M. (2016). Networking in the plant microbiome. *PLoS biology*, 14(2), 1369-1378.

Verscheure, M., Lognay, G., & Marlier, M. (2002). Bibliographic review: chemical methods for the identification and classification of fungi. *Biotechnology, Agronomy and Society Environment*, 6(3), 131–142.

Vincent, G. (2007). Sampling methods for landscape assessment: how to measure storm damage. *L'espace géographique*, 36, 237- 250.

Warcup, J.H. (1950). The soil plate method for isolation of fungi from soil. *Nature*, 166(4211), 117-118.

Yin, M., Xiao, D., Wang, C., Zhang, L., Dun, B., & Yue, Q. (2022). Regulation of BbLaeA on beauvericin and bassiatin production in *Beauveria bassiana*. *World Journal of Microbiology and Biotechnology*, 38(1), 1-10.

Zain, M.E., Awaad, A.S., Al-Othman, M.R., Alafeefy, A.M., & El-Meligy, R.M. (2014). Biological activity of fungal secondary metabolites. *International Journal of Chemical and Applied Biological Sciences*, 1(1), 14-22.