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Diversity and Biological Activities of Mold Isolated from Bilanko and Ngamakala Peat Bog Soils (Republic of Congo)

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Abstract The aim of this work was to characterize molds isolated from Bilanko and Ngamakala peat bog soils in the Republic of Congo. After isolation on Sabouraud medium and morphological characterization, monitoring of enzyme production (protease, amylase, lipase and cellulase) and antagonism capacity were carried out using classical techniques. Enumeration showed a higher fungal concentration at point 6 of Ngamakala site with $9.10\pm2.57.10^3$ versus $5.40\pm1.19.10^3$ CFU/g at point 8 of Bilanko site. 09 fungal genera were identified: *Aspergillus, Trichoderma, Penicillium, Beauvaria, Rhizomucor, Mucor, Onychocola, Fasarium, and Scytalidium*. The Bilanko site was the most diversified with 07 genera, i.e. 52.94%, compared to 04 genera for Ngamakala, i.e. 48.06%, with a predominance of the genus Aspergillus in the 02 sites. 12 isolates (66.66%) produced amylase, protease and lipase, compared to 18 isolates (100%) for cellulase. The genus *Aspergillus* (E2L2-Ngamakala) was the most efficient in lipase production with a diameter of 5.4 cm. The genus *Aspergillus* (E6L3-Ngamakala) was the most invasive isolate on all the other isolates tested with a diameter of 90 mm. These results showed that the peat bog soils at both sites are rich in mold whose biological activities can influence biogeochemical cycles.

Keywords: *antagonism, hydrolases, mold, soil, peat bog*

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1. Introduction

Peat bogs are wetlands that are regularly or constantly saturated with water. Organic matter is only partially decomposed here, due to the low oxygen content, and slowly accumulates to form peat. Wetlands are places where the distinctive soil favors the presence of specialized vegetation that grows in conditions where oxygen is depleted or absent. They are environments with unique terrestrial, hydrological and climatic conditions. In addition to peat bogs, other types of wetlands include marshes, swamps and aquatic grass beds. In these environments, the organic matter produced annually decomposes on site or is transported elsewhere as the water table moves with the seasons [1]. It is the modes of vegetation decomposition and water supply that distinguish peatlands from other types of wetlands. Worldwide, peatlands cover 3% of the world's land area, around 4.632 million km^2, sequester 30% of the total stock of soil organic carbon and are found in 180 countries [2,3]. They are also home to a wide variety of species. The biological functioning of these ecosystems is strongly linked to microbial activity, which gives these micro-organisms a major role in many functions of peatland soils. Studies have shown that general microbiological activity in peat bogs accelerates, leading to strong decomposition of organic matter and significant release of CO_2, due to the rise in temperature as a result of global warming [4]. These environments, which are all the more precious and seriously threatened, are home to significant biodiversity. These include bacteria, micro-algae, archaea, protists and fungi, the existence of which was questionable at the beginning of the 20th century [5]. Microscopic fungi are heterotrophic micro-organisms, divided into two (02) major groups: yeasts and mold. The latter constitute a heterogeneous group of around 20,000 species belonging to four classes: Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes [6,7]. Many mould species produce a wide variety of secondary metabolites, some of which are of vital importance to humans and used in various fields such as agriculture, biotechnology, health and the environment, while others are used for enzyme

production [7]. The Climate change is a threat that could destabilize the entire region. The continued accumulation and preservation of organic matter in a peat bog depends to a large extent on the maintenance of conditions of water saturation, anoxia and reduced activity of microorganisms, particularly molds. Although the study of the diversity of these microorganisms is the object of growing interest, the description of this diversity, the process maintaining this diversity and its role in the functioning and stability of peatlands remains to date very little explored in Congo. It is in this context that our study will focus on the diversity of mold in the soils of the Bilanko and Ngamakala peat bogs, in order to characterize them and assess their impact on the environment.

2. Methods

2.1. Sampling

Peatland soil samples were taken at the Bilanko (Impoh village) and Ngamakala (Lifoula) sites in the Mbé plateau (Pool department). The Mbé plateau is a 6,500 km2 wooded swampy depression occupied by herbaceous savannah, with an altitude of 600 m from Brazzaville to Lefini. Samples were taken randomly from the surface at a depth of 0 to 10 cm. Samples were packaged in sterile vials, labelled and transported to the Cellular and Molecular Biology laboratory of the Faculty of Science and Technology (FST), Université Marien NGOUABI.

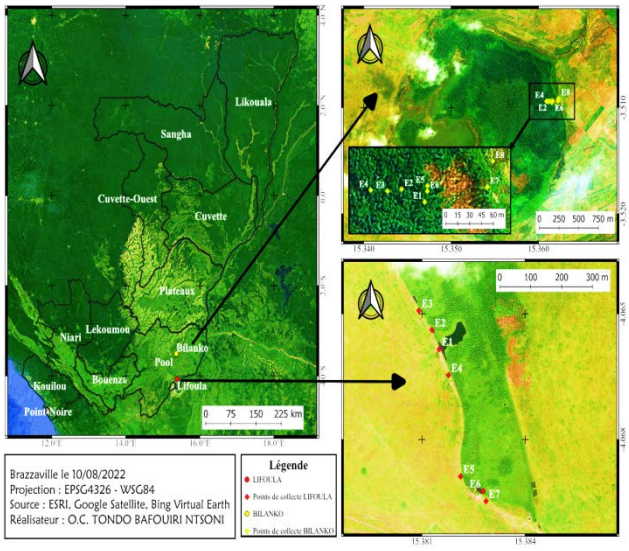

Figure 1. Location of sample collection sites

2.2. Enumeration

2.2.1. Preparation of Dilutions

10g of peat soils from each sample were removed and added to 90 mL of sterile physiological water in an Erlenmeyer flask for a stock solution (SM). Using a pipette, 1 mL of the stock solution was withdrawn and transferred to the marked test tube containing 9 mL of physiological fluid, then mixed to obtain a homogeneous 10-1 solution. Each new solution is ten times less concentrated than the previous one [8].

2.2.2. Inoculation and Culture

Using a pipette, 100µL of each dilution (inoculum) was taken and placed in a Petri dish containing Sabouraud medium, which had previously been poured and solidified. This volume was then spread over the entire dish, and each inoculated dish was cultured in an oven at 37°C for 7 days [9,10].

2.2.3. Observing and Counting Colonies

The technique used to count colonies is surface counting. This gives the number of colony-forming units (CFU) sampled. This method only takes into account viable mold colonies that can develop under the growth conditions used [11].

$$UFC/g = \frac{\text{Number of colonies}}{\text{Dilution factor x Inoculated volume}}$$

2.3. Purification and Storage

The colonies obtained were purified on Sabouraud medium previously poured and solidified in a Petri dish. Plates were incubated at 37°C for 7 days to obtain distinct, homogeneous colonies. Selected isolates were stored at 4°C in Eppendorf tubes containing 200µL glycerol and 800µL liquid medium (LB) [12].

2.4. Macroscopic and Microscopic Identification

Genus's identification was based essentially on the determination keys described in the literature, using pure fungal cultures by phenotypic typing based on observation of macroscopic characteristics of the thallus (shape, color, texture) and microscopic characteristics of the filaments (septum, branching, conidia, conidiophore, sporangiospore). By comparing the characteristic data obtained with those of reference works containing mussel identification keys [12,13,14].

2.5. Biological Activity

2.5.1. Culture Conditions

To assess enzyme production, the preserved isolates were transferred to Sabouraud medium, incubated at 37°C for 48 h and then each colony was inoculated into the Erlenmeyer flask containing 20 mL of liquid LB medium, then incubated at 37°C for 48 h. 4mL of each culture was taken, including 2mL to measure optical density (O.D.), an expression of mold growth, using a spectrophotometer calibrated at 600nm, and 1mL to assess enzyme activity [15,16].

2.5.2. Cellulolytic Activity Evaluation

0.5g cellulose and 1.5g agar were weighed, added to 100 mL distilled water, homogenized and sterilized at 121°C for 15 min. Petri dishes were poured, solidified and wells were made into which 50µL of the supernatant, previously centrifuged at 600/min for 10 min, was deposited. The plates were incubated at 37°C for 18 to 48 hours. After incubation, the Petri dishes were flooded with lugol solution for 30 seconds, then rinsed with distilled

water. The appearance of the clear zone on the cellulose agar plate indicates that the strain has produced cellulase. The diameters of each halo were measured [16,17,18].

2.5.2. Amylolytic Activity Evaluation

1g starch and 1.5g agar were weighed and added to 100 mL homogenized distilled water, then sterilized at 121°C for 15 min. Petri dishes were poured, solidified, wells were made and 50 µL of the supernatant previously centrifuged at 600/min for 10 min was deposited. The plates were incubated at 370°C for 24 to 48 hours. After incubation, the agar medium was coated with Lugol's solution for 30 seconds, followed by rinsing with distilled water. Starch hydrolysis is indicated by the appearance of the translucent zone around the amylase-producing colony. The diameters of each halo were measured [18,19,20].

2.5.4. Lipolytic Activity Evaluation

1mL olive oil and 1.5 g agar were added to 100 mL distilled water, the mixture was brought to the boil and sterilized in an autoclave at 121°C for 20 min. Once the medium had cooled (45 to 500C), it was poured and solidified. Wells were made and 50µL of the supernatant, previously centrifuged at 600/min for 10min, was deposited. The Petri dishes were incubated at 370C for 24h to 48h. Lipid degradation is characterized by visual observation of the clear, transparent zone on the agar after reaction with diluted Lugol's [19].

2.5.5. Proteolytic Activity Evaluation

Milk agar has been used to demonstrate the presence of proteolytic activity in fungal strains [21]. To demonstrate proteolytic activity, 1.5 g of agarose and 10 mL of skimmed milk were placed in 100 mL of sterile distilled water. Petri dishes were poured, solidified and wells were made into which 50µL of the supernatant, previously centrifuged at 600/min for 10 min, was poured for 10 min. The plates were incubated at 370°C for 12 to 18 hours. Degradation of milk casein is characterized by direct visual observation of the clear, transparent zone on the agar [22,23].

2.5.6. Study of Direct Interactions

Antagonism manifests itself either through competition, hyperparasitism, siderophore production or antibiosis [24]. Antagonistic activity has been studied using the direct confrontation method: the opposing cultures technique. This involves placing two agar pellets, one carrying the pure isolate and the other a pure antagonist, in 90 mm Petri dishes containing Sabouraud medium. These explants come from mushroom cultures. Transplants were made at the same time. Incubation was carried out at 370°C for 7 days. Colony diameters of the two antagonistic fungi were measured every day until the seventh day [25].

3. Results

3.1. Mould Enumeration

Figure 2 shows mold colonies on Sabouraud medium after 7 days of cultivation. Colonies of different sizes, shapes and colors can be observed.

Figure 2. mould colonies on Sabouraud medium after 7 days

Table 1. Mould enumeration for Bilanko and Ngamakala samples

Sites	Points	Sabouraud medium
Bilanko	**Point 1**	$(8.00 \pm 2.07).10^2$
	Point 2	$(1.20 \pm 0.34).10^2$
	Point 3	$(1.60 \pm 0.90).10^2$
	Point 4	$(1.70 \pm 0.59).10^3$
	Point 5	$(4.50 \pm 1.09).10^3$
	Point 6	$(3.70 \pm 0.85).10^3$
	Point 7	$(2.10 \pm 0.09).10^3$
	Point 8	$(5.40 \pm 1.19).10^3$
Ngamakala	**Point 1**	$(7.70 \pm 2.15).10^3$
	Point 2	$(8.80 \pm 1.02).10^2$
	Point 3	$(1.30 \pm 0.09).10^2$
	Point 4	$(1.50 \pm 0.34).10^3$
	Point 5	$(3.80 \pm 0.94).10^3$
	Point 6	$(9.10 \pm 2.57).10^3$
	Point 7	$(2.80 \pm 0.84).10^3$

Table 1 shows the fungal loads in the various soil sampling points in the Bilanko and Ngamakala peat bogs. The results show that fungal loads vary according to the site and sampling point. The highest fungal loads were observed at the Ngamakala sampling points compared with those at the Bilanko site, with maximum values of 5.40 ± 1.19.103 CFU/g for the Bilanko site at point 8 and 9.10 ± 2.57.103 CFU/g for the Ngamakala site at point 6 respectively.

➢ **Statistical analysis**

Figure 3 shows the variation in fungal load at the various sampling points on the Bilanko site. There is a significant difference in the fungal load, and a non-proportional distribution of these loads at the different points. Between points 1 and 2 the difference was significant at p = 0.001; between points 2 and 3 it was significant at p = 0.005; between points 3 and 4 it was significant at p = 0.0001; between points 4 and 5 it was significant at p = 0.002 and between points 7 and 8 it was significant at p = 0.013. On the other hand, a non-significant difference was observed between points 5 and 6, and then between points 6 and 7, with values of p = 0.352 and p = 0.137 respectively.

(a= difference between points 1 and 2; b= difference between points 2 and 3; c = difference between points 3 and 4; d = difference between points 4 and 5; e=

difference between points 5 and 6; f = difference between points 6 and 7; g = difference between points 7 and 8)

Figure 4 shows the variation in fungal load at the different sampling points at the Ngamakala site. The results showed a significant variation and a non-proportional distribution of mould loads at the different points of the Ngamakala site. There was a significant difference in mould concentration between points 1 and 2 with p = 0.001; points 2 and 3 with p = 0.0002; points 3 and 4 with p = 0.001; points 4 and 5 with p= 0.022; points 5 and 6 with p= 0.003 and points 6 and 7 with p= 0.001.

(a = difference between points 1 and 2; b = difference between points 2 and 3; c = difference between points 3 and 4; d = difference between points 4 and 5; e= difference between points 5 and 6; f = difference between points 6 and 7).

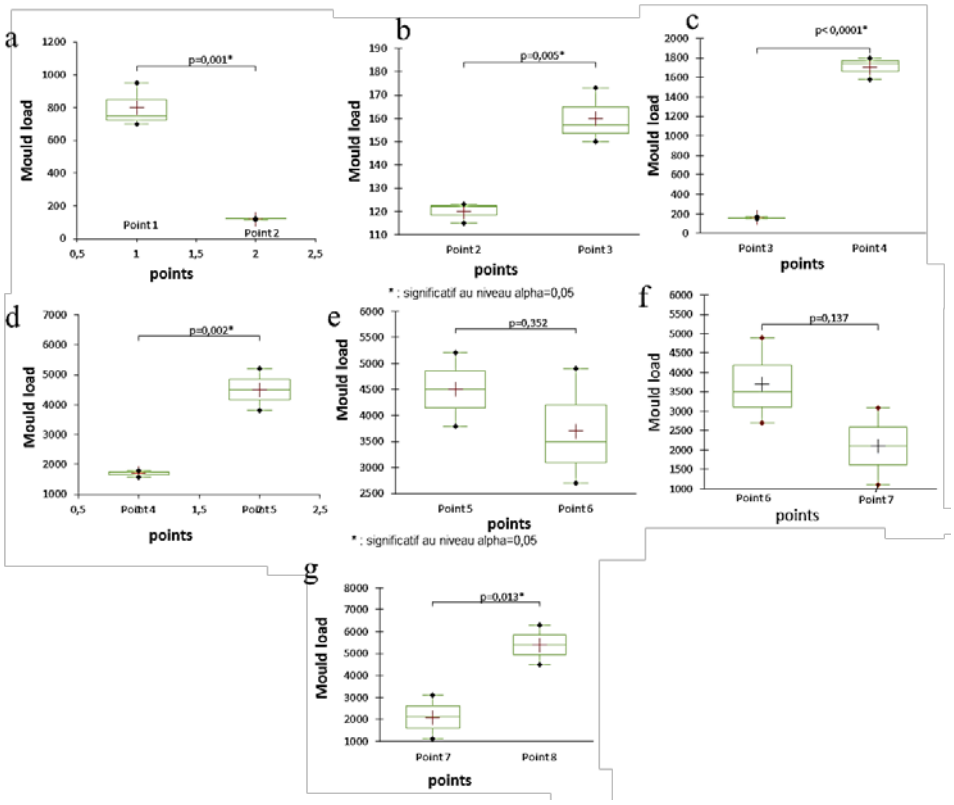

Figure 3. Variation in mould concentration at the Bilanko site

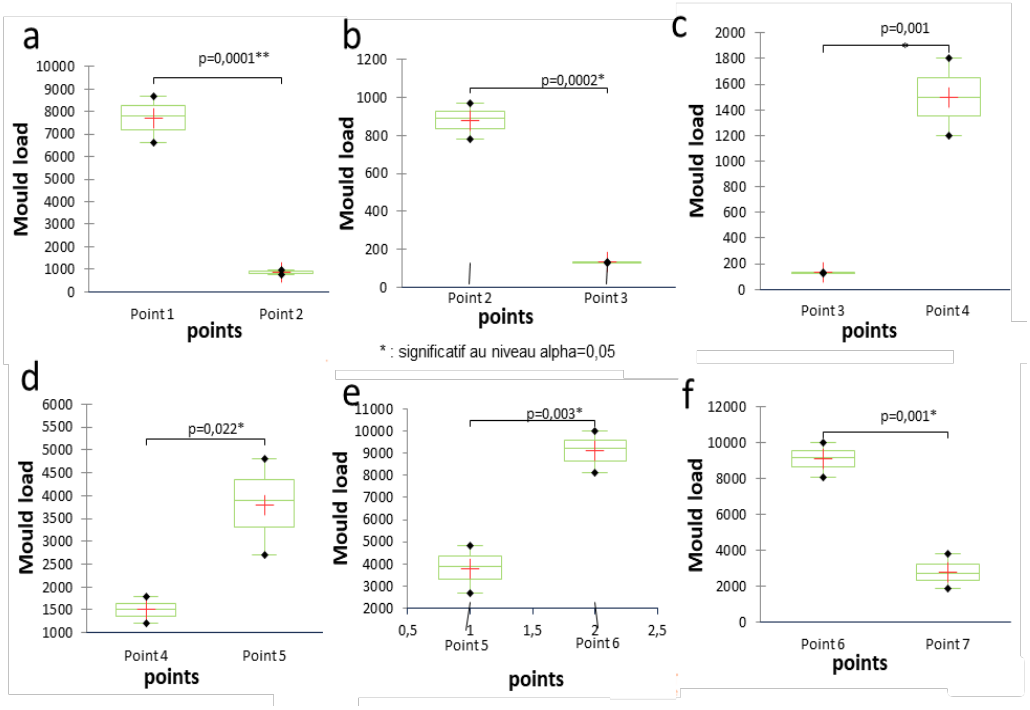

Figure 4. Variation in mould concentration the Ngamakala site

3.2. Purification of Mold

Figure 5 shows the colonies obtained after purification on Sabouraud medium. Isolation revealed a high diversity of mold.

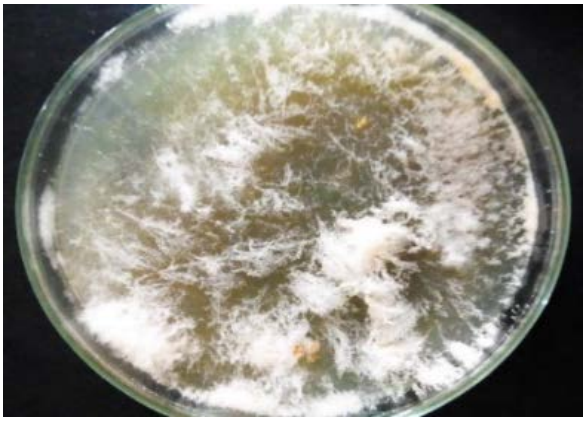

Figure 5. Pure mold isolates on Sabouraud

3.3. Macroscopic and Microscopic Identification

Figure 6 shows the macroscopic and microscopic characteristics of the mold isolated from the peat bog soils at the Bilanko and Ngamakala sites. A total of 34 isolates were obtained, with 18 (52.94%) from the Bilanko site and 16 (48.06%) from the Ngamakala site. 09 mold genera were identified.

Table 2 shows the number and percentage of genera identified at the Bilanko and Ngamakala sites. Seven (7) genera were counted at the Bilanko site: *Aspergillus* with 38.88%; *Trichoderma* and *Penicillium* with 16.66% each; Mucor with 11.11%; *Onychocola*, *Fasarium* and *Scytalidium* with 5.55% each, compared with four (4) genera at the Ngamakala site: *Aspergillus* with 56.25%, *Rhizomucor* with 18.75%, *Beauveria* with 18.75% and *Trichoderma* with 6.25%. The genera *Aspergillus* and *Trichoderma* were found in both sites with respective percentages of 38.88% and 16.66% for the Bilanko site and 56.25% and 6.25% site.

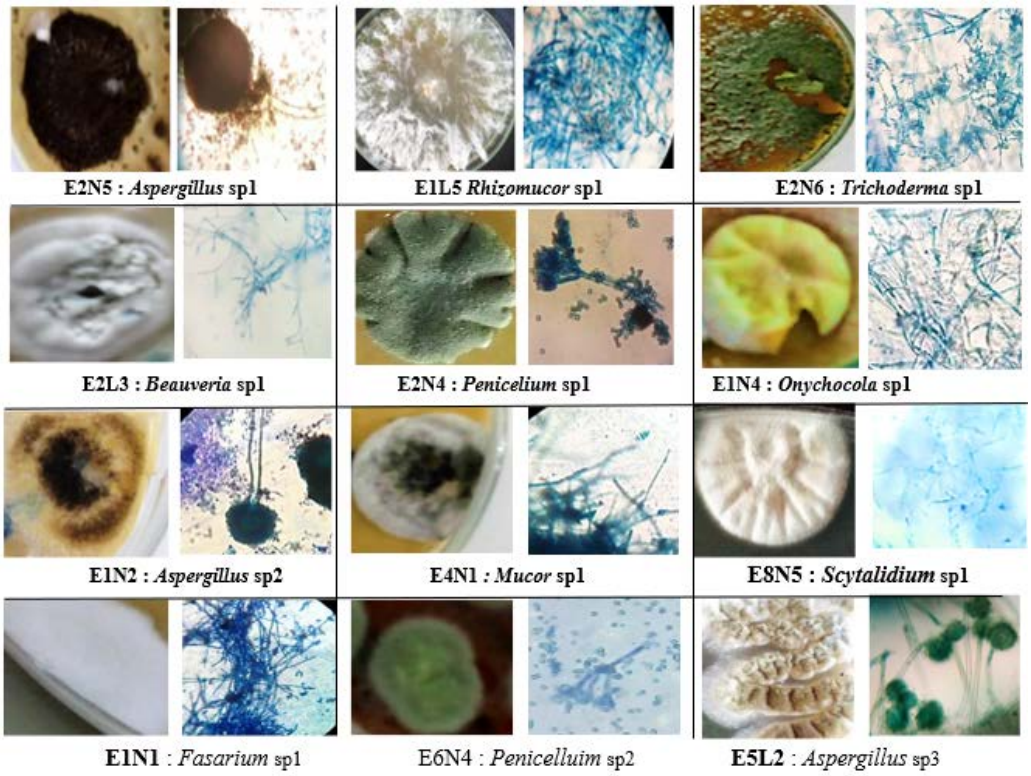

Figure 6. Macroscopic and microscopic characteristics of mold

Table 2. Number and percentage of fungal genera by site

Genus	Bilanko		Ngamakala	
	Number of isolates	**Percentage**	**Number of isolates**	**Percentage**
Aspergillus	7	38,88 %	9	56,25 %
Trichoderma	3	16,66 %	1	6,25 %
Penicillium	3	16,66 %	0	0 %
Beauveria	0	0 %	3	18,75 %
Rhizomucor	0	0 %	3	18,75 %
Mucor	2	11,11 %	0	0 %
Onychocola	1	5,55 %	0	0 %
Fasarium	1	5,55 %	0	0 %
Scytalidium	1	5,55 %	0	0 %
Total	18	52,94 %	16	48,06 %

3.4. Identification of Enzymatic Activities

Of the 34 isolates obtained, 18 isolates were randomly selected for enzymatic activities, including 11 from the Bilanko site and 07 from the Ngamakala site.

3.4.1. Amylolytic Activity

Figure 7 shows the halos indicating starch degradation by the isolates tested. Amylase production is determined to verify the ability of molds to degrade starch. Out of 18 strains tested, 12 showed amylolytic activity.

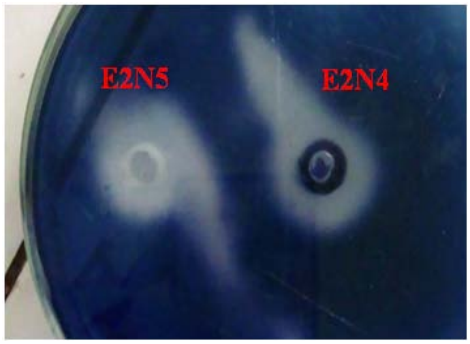

Figure 7. Demonstration of amylolytic activity by isolate (E2N5 *Aspergillus* sp1)

Figure 8 shows the amylase production profile of the isolates. In both sites, the ability to degrade amylase is observed in isolates with an average diameter of 1.9 cm for each. At the Bilanko and Ngamakala sites, these were isolates E2N5 (Aspergillus sp1) and E6L3 (Aspergillus sp13) respectively. The lowest mean diameter value for the Bilanko site is 1.1 cm, and 1.8 for the Ngamakala site. Isolates obtained at the Ngamakala site showed more amylolytic activity.

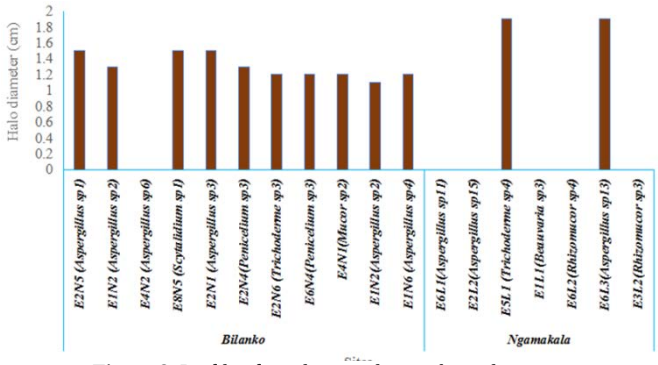

Figure 8. Profile of amylase production by isolates

3.4.2. Cellulolytic Activity

Figure 9 shows halos of cellulose degradation by the isolates tested. Our results showed that all isolates possessed cellulolytic activity, i.e. a percentage of 100%.

Figure 10 shows the variation in cellulase production halos for the different isolates. At the Ngamakala and Bilanko sites, the average diameters obtained range from 1.5 cm for E1N2 (*Aspergillus* sp2) to 3.8 cm for isolate E4N2 (*Aspergillus*); and from 1.4 cm for E1L1 (Beauveria sp3) to 1.8 cm for isolate E6L3 (*Aspergillus* sp13).

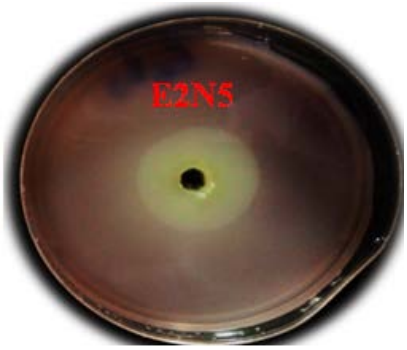

Figure 9. Evidence of the cellulolytic activity of the isolate (E2N5 *Aspergillus* sp1)

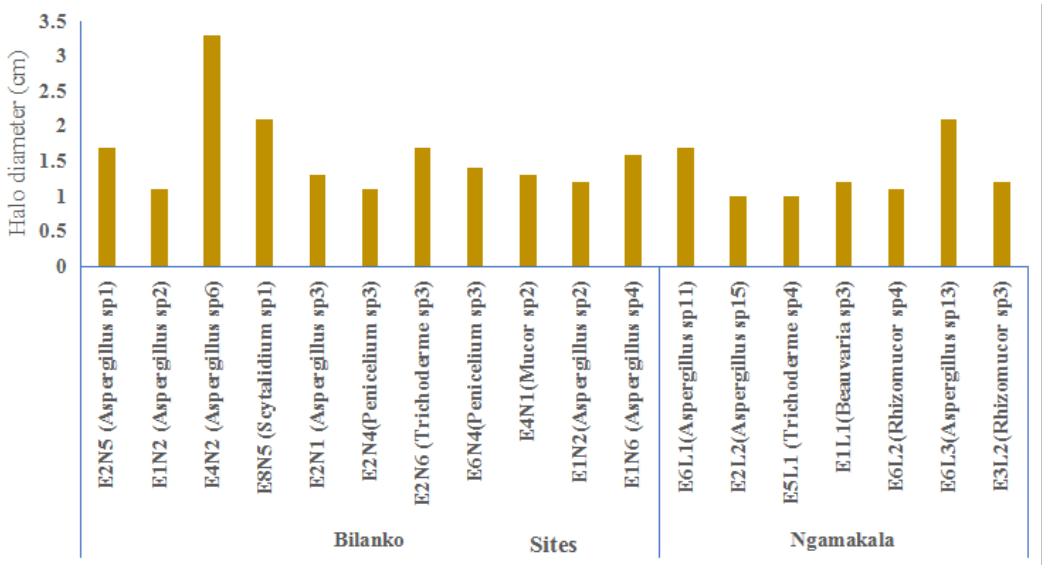

Figure 10. Cellulase production profile of different mold isolates

3.4.3. Proteolytic Activity

Figure 11 shows the halos indicating the digestion of casein from skimmed milk. The results obtained demonstrate the ability of molds to degrade proteins perfectly. All 18 isolates used showed proteolytic activity, with halo diameters ranging from 07.3 to 2.1 cm.

Figure 12 shows the protease production profile of different isolates. Average diameters for protease production ranged from 2.1 cm E1N2 (*Aspergillus* sp2) to 7.2 cm E2L2 (*Aspergillus* sp15) for both sites.

3.4.4. Lipolytic activity

Figure 13 shows lipase production by the isolates. The ability of molds to produce lipases was tested positive with 17 out of 18 isolates.

Figure 14 shows the lipase production profile of the various isolates tested. The molds found at the Ngamakala site showed impotent lipolytic activity, with mean halo diameter values ranging from 1.1 cm for isolate E6L2 (*Rhizomucor* sp4) to 5.4 cm for isolate E2L2 (*Aspergillus* sp15).

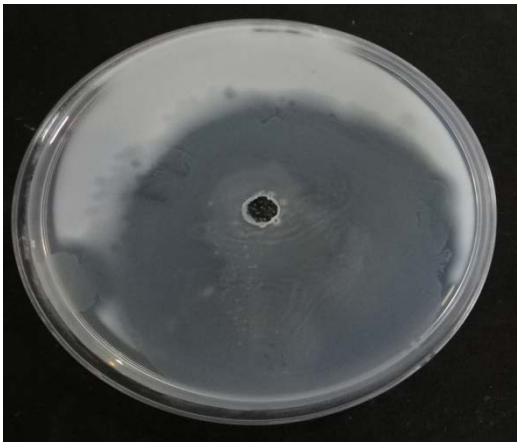

Figure 11. Evidence of proteolytic enzyme production by isolates

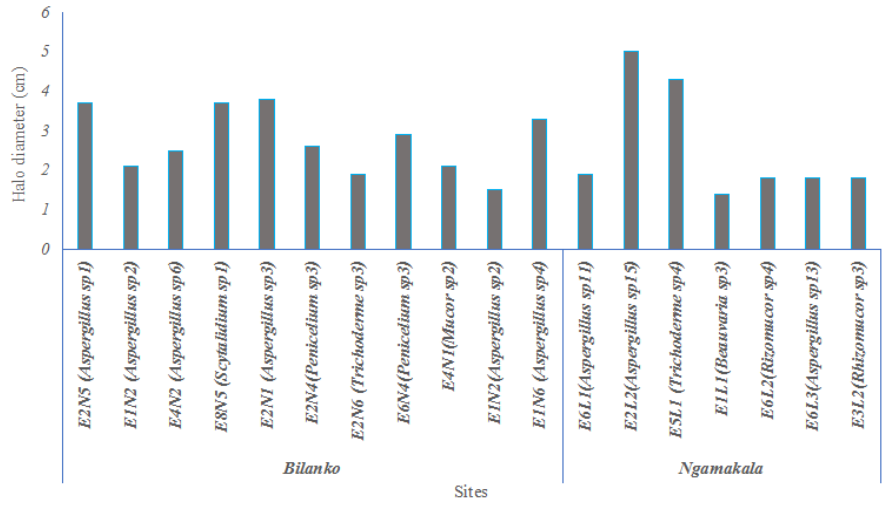

Figure 12. Protease production profile of different mold isolates

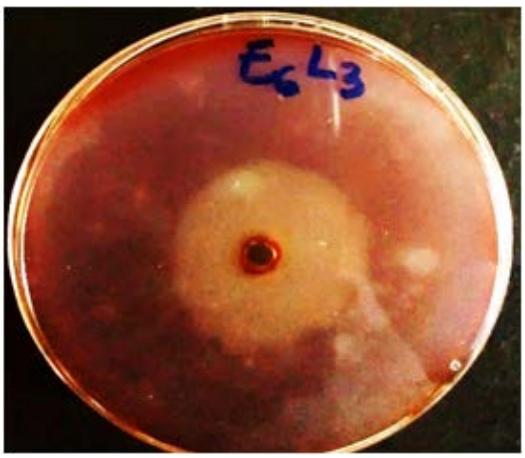

Figure 13. Demonstration of lipase production by isolates

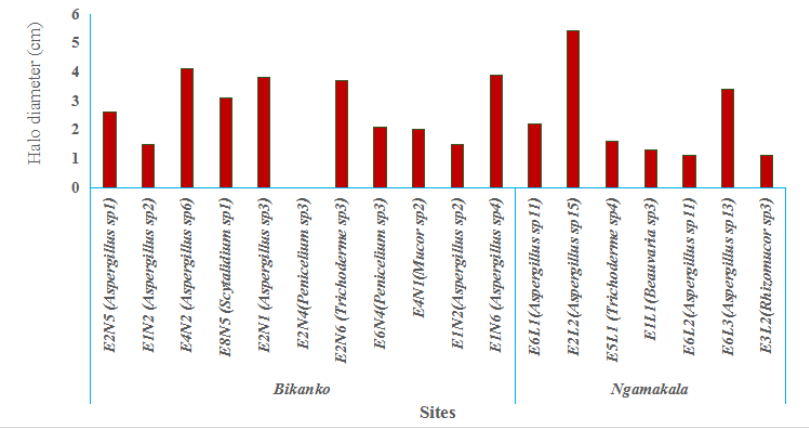

Figure 14. Lipase production profile for different isolates

3.5. Antagonism Test

Biological control of fungal isolates was carried out by antagonism testing, with strains selected randomly according to genus. The effectiveness of antagonistic control depends on the isolate's ability to grow rapidly.

➢ E6L2 (*Rhizomucor* sp4) and E6L3 (*Aspergillus* sp11)

Figure 15 illustrates the direct confrontation between E6L2 (*Rhizomucor* sp4) and E6L3 (*Aspergillus* sp11). The antagonism test between E6L2 and E6L3 showed that the E6L2 isolate covered the entire dish, while the E6L3 isolate did not. This inhibits mycelial growth, as the E6L3 isolate grown alone occupies a larger surface area than that obtained by the test.

Figure 16 shows variations in the diameters of E6L2 and E6L3 isolates in direct confrontation after 6 days. From day 5, isolate E6L2 occupied almost all of the 90 mm on the entire surface of the dish, whereas isolate E6L3 occupied only 17 mm up to day 6.

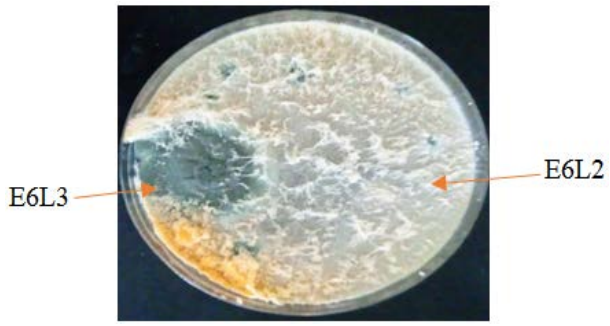

Figure 15. Direct comparison between E6L2 (*Rhizomucor*) and E6L3 (*Aspergillus*)

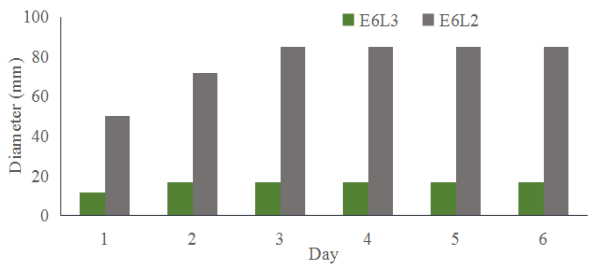

Figure 16. Growth trends of E6L2 and E6L3 isolates in direct comparison

➢ E6L3 (*Aspergillus* sp6) and E2N5 (*Aspergillus* sp1)

Figure 17 shows the direct comparison between isolates E6L3 and E2N5, all belonging to the *Aspergillus* genus. The E6L3 isolate occupied the entire surface of the dish, while bypassing the E2N5 isolate, whose growth stopped as soon as it came into contact with E6L3.

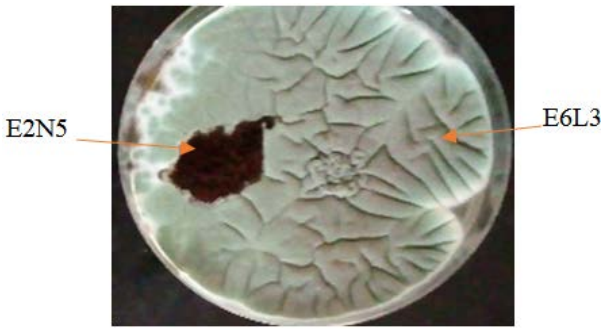

Figure 17. Direct confrontation between E6L3 (*Aspergillus*) and E2N5 (*Aspergillus*)

Figure 18 shows the growth of E6L3 and E2N5 isolates in direct confrontation. After 4 days of incubation, the E6L3 isolate (*Aspergillus* sp6) had completely invaded the dish, while the growth of the E2N5 isolate was inhibited and its diameter did not exceed 23.33 mm until day 6.

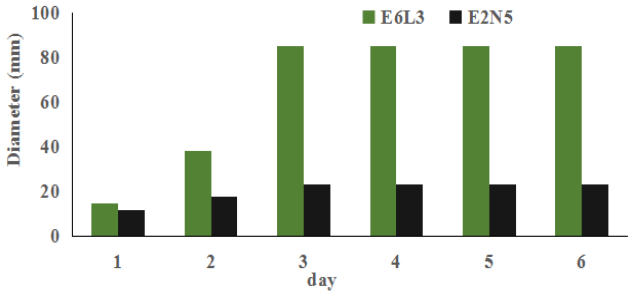

Figure 18. Growth trend of E6L3 and E2N5 isolates in direct comparison

➢ E1N6 (*Aspergillus* sp4) and E4N3 (*Beauveria* sp1)

Figure 19 shows the direct comparison between isolate E1N6 belonging to the genus *Aspergillus* and isolate E4N3 (genus *Beauveria*). The fast-growing E1N6 isolate invaded the entire dish without coming into contact with the slow-growing E4N3 isolate.

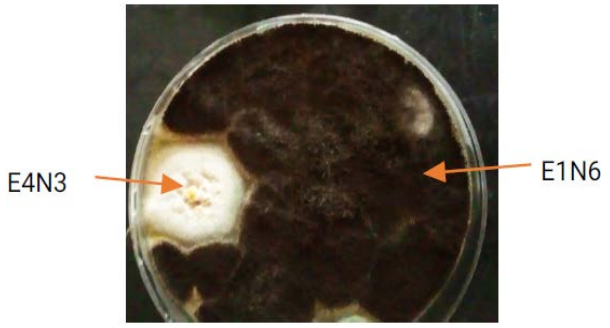

Figure 19. Direct confrontation between E1N6 (*Aspergillus*) and E4N3 (*Beauveria*)

Figure 20 shows the growth of E4N3 and E1N6 isolates in direct confrontation over 6 days. From day 1 to day 3, the growth of both isolates increased proportionally. After 4 days of incubation, isolate E1N6 began to invade the whole box, but did not touch isolate E4N3, whose growth did not exceed 26.33mm. The two mold remained distant from each other until day 6, despite the rapid growth of isolate E1N6.

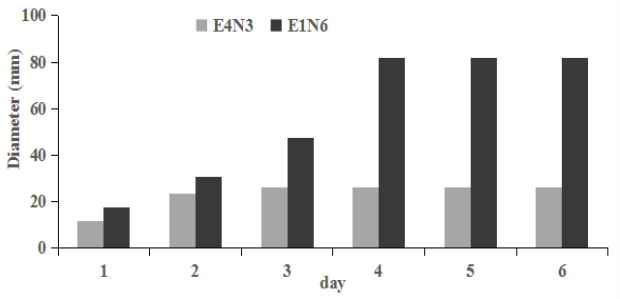

Figure 20. Growth trend of E4N3 and E1N6 isolates in direct comparison

➢ E1N5 (Aspergillus sp8) and E1L1(Beauveria sp3)

Figure 21 shows the direct comparison between *Aspergillus* isolate E1N5 and *Beauveria* isolate E1L1. It can be seen that, while both isolates grew over the days, they remained distant from each other.

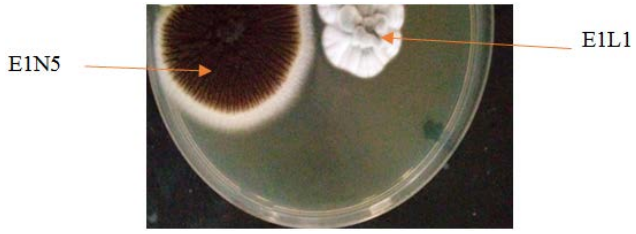

Figure 21. Direct comparison between E1N5 (*Aspergillus*) and E1L1 (*Beauveria*)

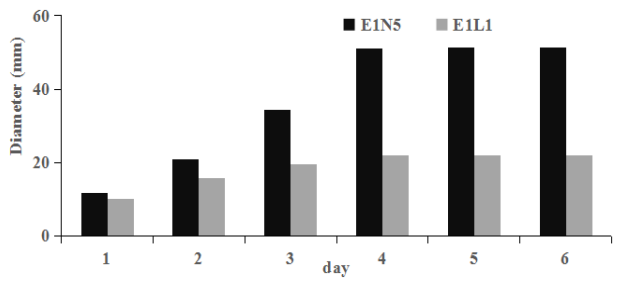

Figure 22. Diameter trends for E1N5 and E1L1 isolates

Figure 22 shows the variation in growth diameters of E1N5 and E1L1 isolates as a function of time (days). Up to the 6th day of incubation, the two molds tested remained distant from each other despite their growth, with the largest values of 51.33mm and 22mm respectively.

➢ E4N4 (*Penicelium* sp1) and E2N5 (*Aspergillus* sp1) isolates

Figure 23 shows the direct comparison between isolates E4N4 of the genus *Penicelium* and E2N5 of the genus *Aspergillus*. Both isolates develop without inhibiting each other's growth until day 7.

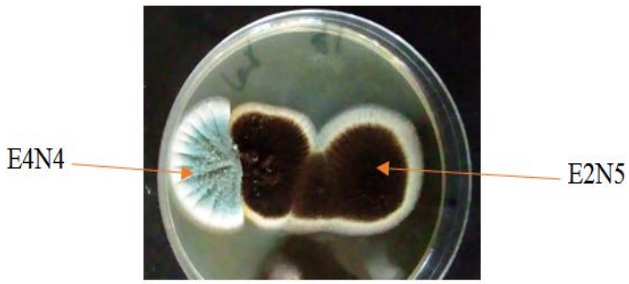

Figure 23. Direct comparison between E4N4 (*Penicelium*) and E2N5 (*Aspergillus*)

Figure 24 shows the evolution of the growth diameters of E4N4 and E1L1 isolates as a function of time (days). After 6 days, the diameters of the isolates evolved until they came into contact with each other.

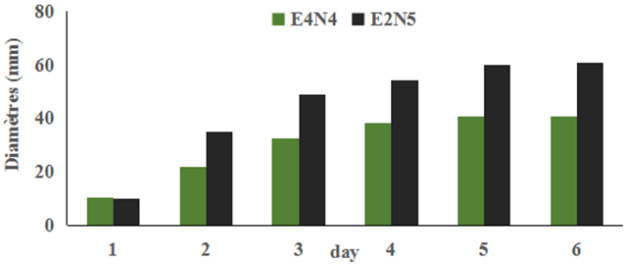

Figure 24. Growth trend of E4N4 and E1L1 isolates in direct comparison

➢ E3L3 (*Trichoderma* sp4) and E1L1(*Beauveria* sp3)

Figure 25 shows the direct comparison between E3L3 and E1L1. The two isolates tested developed independently until they came into contact. Isolate E3L3 grew faster than isolate E1L1.

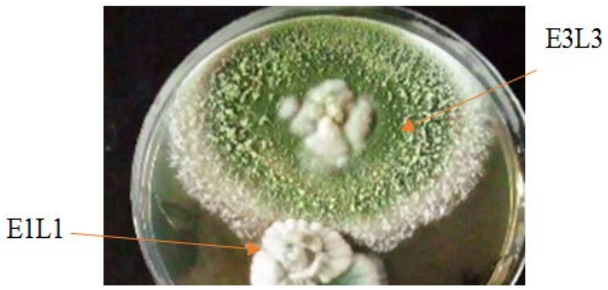

Figure 25. Direct comparison between E3L3 (Trichoderma) and E1L1 (Beauveria)

Figure 26 shows the evolution of the diameters of E3L3 and E1L1 isolates as a function of time (days). Up to day 6, these isolates grew to a diameter of 80.66mm for E3L3

and 25mm for E1L1.

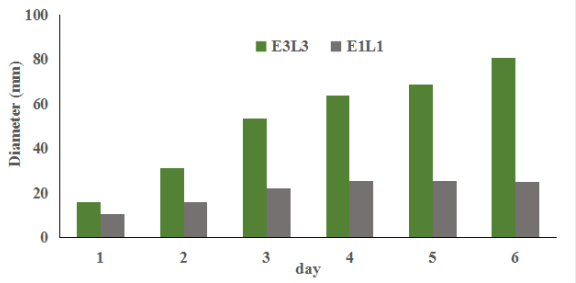

Figure 26. Evolution of E3L3 and E1L1 isolates in direct comparisons

4. Discussion

The aim of this study was to characterize the mold isolated from the soils of the Bilanko and Ngamakala peat bogs. Soil is a reservoir of various microorganisms, depending on the method used. As highlighted in the previous section, peatlands have a high fungal diversity, with growing interest in peatland functioning and climate change. This is explained by the environmental conditions that govern peat ecosystems.

Mould counts on Sabouraud culture medium showed a varied fungal flora at 2 sampling sites. At the Bilanko site, fungal concentration ranged from $(1.20\pm0.34).10^2$ to $(5.40\pm1.19).10^3$ CFU/g, compared with $(1.30\pm0.09).10^2$ to $(9.10\pm2.57).10^3$ CFU/g at the Ngamakala site, depending on the sampling point. These results show that the Ngamakala site has a very abundant population compared with the Bilanko site. Statistical analysis of mold load variations between the various sampling points revealed significant differences between points and between sites. There was also a non-proportional distribution of fungal loads between the different points. This difference could be explained by the variation in physico-chemical parameters from one point to another [30].

After using the phenotypic identification key, 09 genera were identified: *Aspergillus; Trichoderma; Penicillium; Beauveria; Rhizomucor; Mucor; Onychocola; Fasarium; Scytalidium*. These results are in line with those obtained by Barjac [26] and Waksman and Stevens [27], who assert microbial diversity in the surface layer of peat bogs. In both sites, the genus Aspergillus remains the most represented genus, as shown by Labiod and Chaibras on the isolation of molds from a forest soil in Constantine (Algeria), with a frequency of the genus *Aspergillus* of 50% [10]. Our results also concur with those of Abdlaziz, where the genus *Aspergillus* was in the majority with a frequency of 37.5%, grouping together 6 different species belonging to the genus *Aspergillus*, from arid soil [28].

These fungal genera are present in the majority of soils of all kinds; Alvarez-Rodriguez et al. and Boiron have stated that the genera: *Aspergillus, Penicillium, Fusarium, Trichoderma, Mucor*, are autochthonous strains, usually isolated from most soils [6,29]. The number and activity of these populations has been shown to vary from region to region, and may be influenced by soil organic matter content, soil texture, pH, moisture, temperature, aeration and other factors [31].

In order to better understand the involvement of molds in peatification, the production of four enzymes was demonstrated: amylases, cellulases, proteases and lipases. The results showed that all 18 isolates tested were capable of producing at least three (03) enzymes. The enzymatic activity of microorganisms is directly influenced by the availability of nutrients in the environment, and is part of the adaptation of these microorganisms to their natural environment. Protease production was tested positive with all isolates. According to [32,33], proteases are the most important enzymes that can be produced by several fungal genera such as *Trichoderma, Mucor, Rhizomucor*, etc. These results allow us to consider these isolates as producers of exocellular proteases [34]. Cellulose degradation was observed in all isolates tested. This can be explained by the fact that the rate of cellulose decomposition depends on the microorganisms involved and the ecological conditions. These results are in line with work carried out by [35,16], which demonstrated the ability of fungal genera, notably *Trichoderma*, to produce cellulase. With regard to amylase production, 12 isolates were able to degrade starch. This ability of fungal isolates to degrade starch has already been demonstrated in a study by Tatsinkou and colleagues [19], who showed the ability of certain fungal strains to produce alpha amylase. Lipase production was most significant in isolates from the Ngamakala site, which showed significant lipolytic activity, with mean halot diameters ranging from 5.4 cm for *Aspergillus* isolate E2L2 to 1 cm for *Rhizomucor* isolate E6L2.

The results of the antagonist test show that there are different antagonistic effects with the isolates tested. Studies by Laclere [36]; Vannacci and Herman, [37] have shown that many fungi produce compounds with properties that can interfere with the growth and activity of other fungi. Direct confrontation between isolates E6L3, E1N6, E1N5 and E1N6, all belonging to the genus *Aspergillus*, and isolates E2N5, E4N3, E1L1 and E6N4 respectively, showed a reduction in mycelial growth in the colonies of the latter. Indeed, the genus *Aspergillus* is renowned for its ability to produce inhibitory molecules against other fungal strains. Seidl and colleagues [38] have confirmed this by comparing *Aspergillus* niger with other fungal strains. E6L2 of the genus *Rhizomucor*. Benmechirah and Lidjici [39], working on the antagonistic activity of molds, have demonstrated the inhibition of Fasarium solani mycelium growth in relation to *Aspergillus* niger. This inhibition of fungal growth may be due to exponential growth leading to the secretion of harmful extracellular compounds such as xylanases, enzymes that degrade the cell wall. In the case of direct confrontation between isolates E6L2 and E6L3, we can see that isolate E6L2 is growing rapidly and has covered isolate E6L3. It does not allow the latter to grow below the colony at the same time [40].

5. Conclusion

This work phenotypically characterized molds in Bilanko and Ngamakala peat bog soils, their enzymatic production and antagonism. Results showed a richness of molds in both sites with high fungal loads, 09 genera identified: Aspergillus; Trichoderma; Penicillium;

Beauvaria; rhizomucor; Mucor; Onychocola; Fasarium and Scytalidium. Good enzyme production was observed with lipase diameters of 5.4 cm with Aspergillus (E2L2-Ngamakala) and strong antagonistic activity with 90 mm diameter with the genus Aspergillus (E6L3-Ngamakala). The Bilanko and Ngamakala peat bogs contain a diversity of molds capable of producing enzymes and inhibitors.

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