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EFFICACY OF A PHYTO-PURIFICATION LAGOON USING FUNGAL MYCO-FLORA IN NORTH EAST ALGERIA

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ABSTRACT

The wastewater treatment plant of the Beni Fouda region Willaya of Setif Algeria is a natural lagoon wastewater treatment plant based on the phyto-purification technique which is less expensive, simpler to operate and environmentally friendly, resulting from a set of physico-chemical and biological reactions carried out by plants and micro-organisms on the pollutants present in these waters. The main objective of this research is to evaluate the diversity of fungal myco-flora of the roots of *Phragmites australis* in the city's wastewater treatment plant. The results of the isolation of fungal flora from the roots of the reed purifying plant "*Phragmites australis*" reveal a high diversity of fungal myco-flora and 11 genus are counted. The most dominant genus during the study period was *Trichoderma* sp with a percentage of 23%, followed by the genus *Fusarium sp* with a percentage of 18%. The diversity indices show that the distribution of the myco-flora genus varies according to the sampling season and the level of the stratum where the waters rich in nutrients and mineral salts are found.

KEY WORD : Diversity, Myco-flora, *Phragmites australis*, Sewage water, Wastewater treatment plan, Beni fouda, Algeria.

INTRODUCTION

Pollution of surface and ground water is possible through domestic and industrial wastewater discharges as well as through the use of fertilizers and pesticides in agriculture. Wastewater treatment is the process applied with the aim of reducing the quantity of pollutants in order to reach the standard of effluent discharge into the natural environment or to reuse water. Wastewater treatment returns to the natural environment water that is far from being pure, but which brings the least danger and can preserve the characteristics of ecosystems, such as the application of phyto-purification in wastewater treatment.

The purifying potential of aquatic plants and more particularly reeds was highlighted by Seidel as early as 1946 to treat effluents (Abissy and Mandi, 1999). These plants have the particularity of being tolerant to pollutants and capable of degrading them (Arienzo, 2009). Reed plants are a shelter for bacteria, algae and zooplankton; they contribute to the diversification and balance of biological activity (ONA, 2014). They exploit the capacities of root systems to adapt to high pollution loads and to the conditions of anoxia or hypoxia of the substrate, leading to symbiotic relationships between microorganisms and roots that promote the elimination of pollutants (Kern and Idler, 1999), the fungal communities of healthy reed roots were the ten most diverse plant species (Packer, 2017).

Phragmites australis is facultatively mycorrhizal (Oliveira, 2001) and is also colonized by endophytes (in plant tissue) and epiphytes (on plant tissue) are considered to be at least partly mutualistic (Harley and Harley, 1987; Ernst, 2003).

The objective of this research is to estimate the fungal flora of reed roots (*Phragmite australis*) in the sewage treatment plant of the commune Beni Fouda Wilaya of Sétif: a semi-arid influenced zone, and to evaluate their biodiversity during the study period.

MATERIALS AND METHOD

Study Area

The natural lagoon station is located 1 km east of the town of Beni Fouda and 30 km from the town of Sétif (North-East, Algeria). The geographical coordinates are 36° 17'98 '' N and 5° 36'56 '' E and 833 m altitude. The lagoon station was created in 2006. Its urban wastewater treatment capacity is 11,200 Eq/inhabitant and the daily treatment flow is 1,341 $m3d^{-1}$ (Chennafi, 2018). The treatment is ensured by a long residence time in several basins in series. This system comprises 6 lagoon series with three tiers in tiers. Wastewater treatment by biological process; uses reed-type phyto-purifying plants (Phragmites australis) and massette (*Typha latifolia*) downstream for finishing treatment. It is planned to protect the Wadi Dheb which converges towards the Beni-Haroun Dam (ONA, 2011).

Methodology for Fungal Flora Analysis

Sampling was conducted seasonally (winter and spring) on roots of *Phragmite australis* found at lagoons 03, 05 and 06 and during the year 2018 (Figure 01).

The samples were placed in paper bags for analysis in the laboratory. After drying at 45 °C, small fragments of reed roots are cut off and disinfected with 2% sodium hypochlorite for 2 minutes, then rinsed three times with sterile distilled water for 2 minutes each time. The fragments are dried under aseptic conditions and subsequently placed in petri dishes containing Potato Dextrose Agar plus antibiotic (PDA+) medium. Incubation of the plates is done for 7 days at 24°C.

For each plate the overall colony count is determined. Colonies with similar morphology are grouped into subgroups.

Purification is performed by subculturing the different colonies with a Pasteur pipette; by placing a 6 mm diameter disc in the center of the petri dish

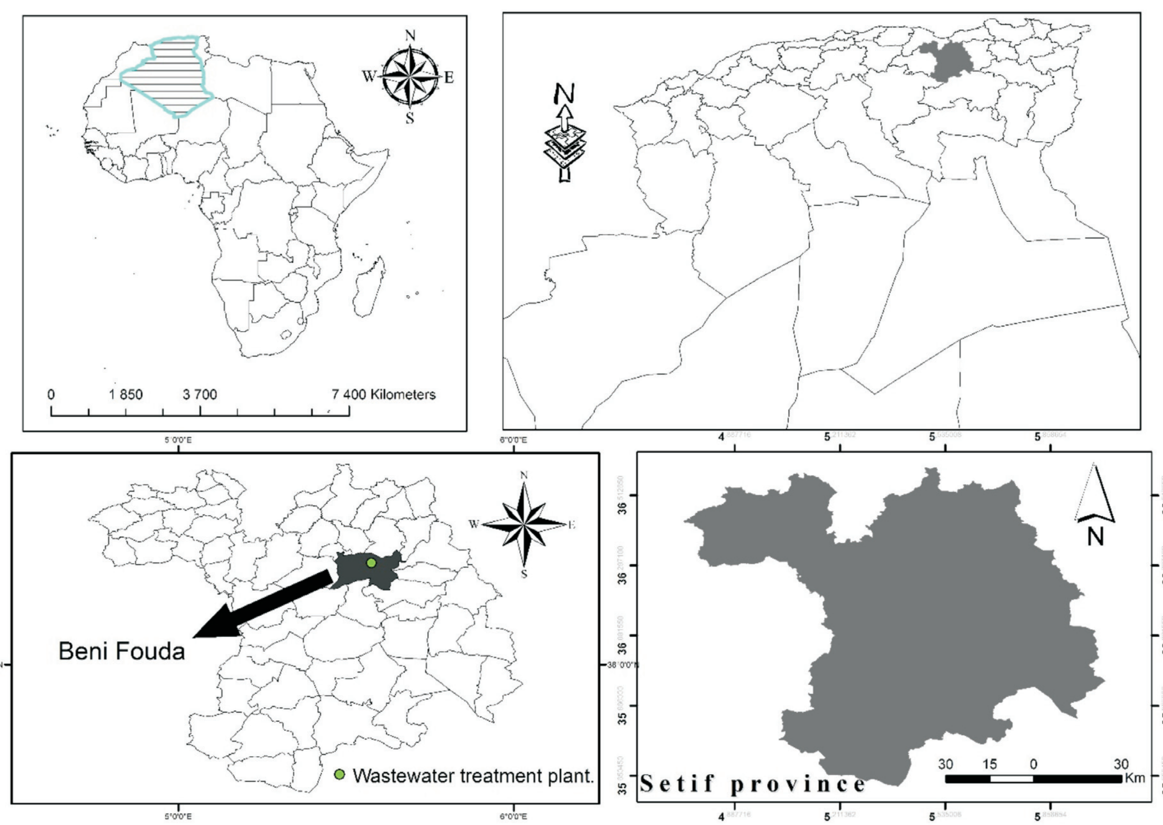

Fig. 1. Geographical Location of the WWTP of Beni Fouda and the sampling lagoons.

containing PDA medium, the dishes are then incubated at 28 ± 4 °C for 7 days. The identification of fungal genus is based on morphological characters (colony appearance, color and sporulation) and microscopic characters (mycelium and spore appearance).

RESULTS AND DISCUSSION

Study of the fungal flora

The results of the isolation of fungal flora from the roots of reeds "*Phragmit australis*" reveal an important diversity of fungi over the sampling seasons. A total of 86 isolates were recorded of which 55 isolates in the vernal period belonging to 10 different genus and 31 isolates in the winter period consisting of 5 genus. Figure 2 shows the number of isolates of fungal genus obtained as a function of the seasons.

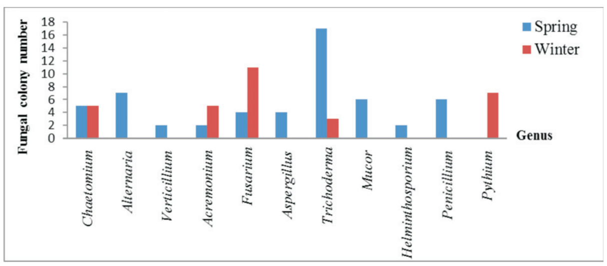

Fig. 2. Comparison of the number of colonies obtained by genus in the winter and spring seasons.

The difference in the number of isolates per genus obtained differs by season. Indeed, during the winter period the number of fungal genus noted is higher and more diversified compared to the spring period.

We identified isolates belonging to ten genus in the spring period of which the majority genus is: Trichoderma with a percentage of 31%, followed by the genus *Alternaria* with 13%, then the genus of *Mucor, Penicillium* with 11%, the genus *Chaetomium* with 9%, the genus *Aspergillus, Fusarium* with 7%, the genus *Helminthosporium* , *Acremonium* with a low percentage of 4% and the genus *Verticillium* with a very low percentage of 3% (Fig. 3).

Isolations revealed only five genus in the winter samples. The genus *Fusarium* is dominant with 35%, followed by the genus *Pythium* with a percentage of 23%, then the genus *Chaetomium, Acremonium* with a percentage of 16% for each genus and finally the genus *Trichoderma* with a low percentage of 10% (Fig. 4).

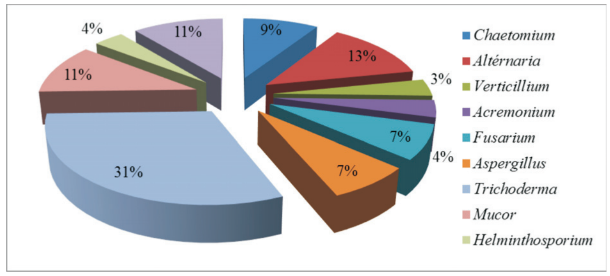

Fig. 3. Percentages of genus isolated in spring

A summary of the isolations made for the two

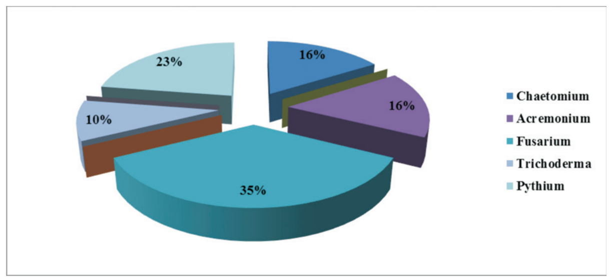

Fig. 4. Percentages of genus isolated in winter.

seasons reveals the dominance of the genus *Trichoderma* with a percentage of 23%, followed by the genus *Fusarium* with a percentage of 18% (Fig. 5).

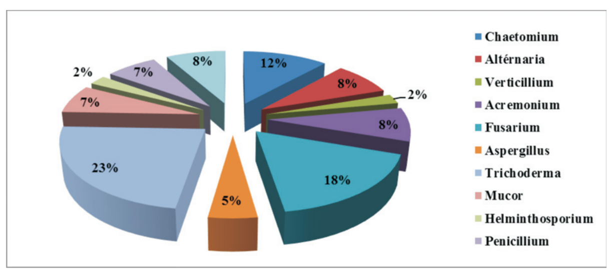

Fig. 5. Percentages of genus for the two winter-spring seasons

Trichoderma species are known for their rapid growth and ability to use different substrates and are, therefore, the major element in terrestrial and marine mycoflora (Widden and Abitrol, 1980; Kubicek, 2003).

Some *Trichoderma* species are bioremediating agents, capable of assimilating heavy metals (Akhtar *et al.,* 2009). This indicates that the presence of this genus at the roots of phragmite australis helps to improve its phyto-purifying function.

Merzoug *et al.* (2018) reveals the presence of the genus *Trichodrma* with a percentage of 12.45%

among other fungal isolates in the olive rhizosphere showing symptoms of root decline and rot caused by the genus *Fusrium* sp.

The genus *Fusarium* is a broad and variable genus that includes saprotrophic fungi found on decaying plant material or parasites of higher plants (Barnett and Hunter, 1998).

According to Dossa. (2019), *Fusarium* pathogens of cultivated plants, including *Fusarium oxysporum*, are species of phytopathogenic fungi found in cultivated soils in both temperate and tropical regions. Van Ryckegem and Van Ryckegem and Verbeken (2005), show the existence of *Fusarium* among several other fungal genus at the level of phragmite australis from different environments.

The diversity of the fungal flora of reed roots is variable according to the seasons and the nutrient composition of the water. The water is rich in mineral and nutritive elements, which is why the fungi find a favourable environment for their development.

The presence of phyto-purifying plants, such as *Phragmit australis*, has an influence on the diversity of microorganisms. Indeed, root development increases the surface area for the development of microorganisms. Root tissues and their exudates probably constitute more welcoming niches for microorganisms than inert mineral substrates. The natural lagoon can be considered as a trap for the biodiversity of different microorganisms.

Biodiversity indices

According to the Table. 1 and Figure 6; the index of diversity (Shannon) and equitability in the Beni Fouda station during the study period showed that the spring season has a higher species richness with nine genus. Thus, the result shows that the diversity of genus of the mycoflora varies according to the season.

The number of genus of mycoflores is greater in the spring season than in the winter. This diversity can be explained by the environmental conditions that are very favourable for the development and variation of the genus in the spring season, whereas in winter, a decrease in species richness due to unfavourable conditions for the development of the majority of mycoflores can be observed (Fig. 7).

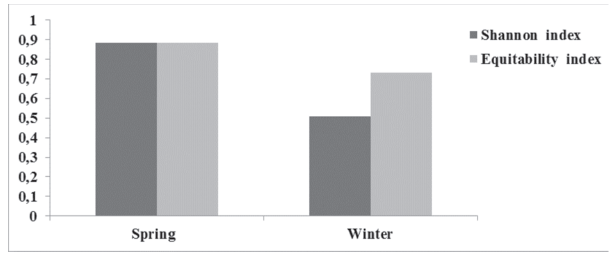

Fig. 6. Shannon-Weaver index (H) and Pielou equitability index (E) for two seasons

In order to evaluate the diversity of mycoflora between the sampling basins, the spring season was chosen as it is more favourable for the development of fungal genus (Figure 07).

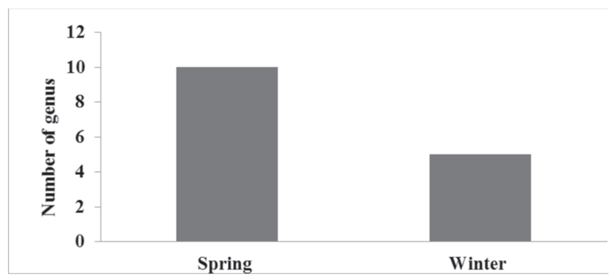

Fig. 7. Variation in the number of the genus of mycoflora as a function of season.

According to Table 02 and Figure 08 and 09 below; the diversity index (Shannon) and the equitability index of Pielou (E) in the three basins of the studied station showed that basin 05 and 06 have a similarity of abundance, except for basin 3 which showed a high specific richness with six genus. This specific richness varies according to stratum, it is low in the maturation basins (05 and 06) where the wastewater is purified ensuring a decrease in pollution, therefore the decrease in organic and mineral nutrients, causes the decrease in the richness of the mycoflorous genus in the last two basins of the station. Consequently the quality of the water influences the biodiversity of the fungi.

Table 1. Shannon's index and equitability as a function of seasons.

Indices	Studied stations	
	Spring	Winter
Shannon Index H	0.88	0.51
Equitability Index E	0.92	0,84

Table 2. Shannon and equitability indexes for three basins in the spring season.

Layer	Basin 3	Basin 5	Basin 6
Shannon Index H	0.74	0.35	0.53
Equitability Index E	0.95	0.58	0.88

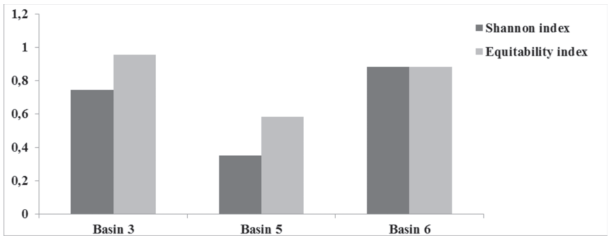

Fig. 8. Shannon-Weaver index (H) and Pielou equitability index (E) of three lagoon basins in the spring season.

Table 3. *Mycoflora* genus counted in three basins of the lagoon studied.

Genus	Basin 3	Basin 5	Basin 6
Chaetomium	+	-	-
Altérnaria	+	-	+
Verticillium	+	-	-
Acremonium	+	-	-
Fusarium	+	-	-
Aspergillus	+	-	-
Trichoderma	-	+	+
Mucor	-	+	-
Helminthosporium	-	+	+
Penicillium	-	+	+
Total	6	4	4

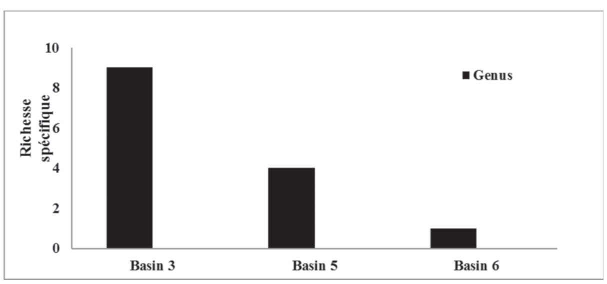

Fig. 9. Specific richness of mycoflora genus in the three basins of the lagoon studied in the spring season.

CONCLUSION

Mycoflores analyses of the roots of the plant type *Phraghmite australis* of the studied station show that there is a great mycological diversity which varies according to time and stratum and well according to the quality of the waste water. The study of the ecological indices has allowed us to conclude that there are so-called constant genus since they occur in the two different seasons studied: cases of *Cheatomium sp, Acremonium sp, Fusarium sp, Trichoderma sp*, and the remains with accidental appearance with maximum and minimum percentages according to the existence of climatic conditions favorable to their development. The indices of diversity and specific richness confirm that a great variation in the genus of mycoflorea is marked during the spring season and in the stratum where the waters are rich in nutrients and mineral salts where they can find their nutritional needs. The climatic conditions and the quality of the wastewater have a considerable influence on the diversity and abundance of the mycological genus of the phyto-purifying roots *Phragmites australis*.

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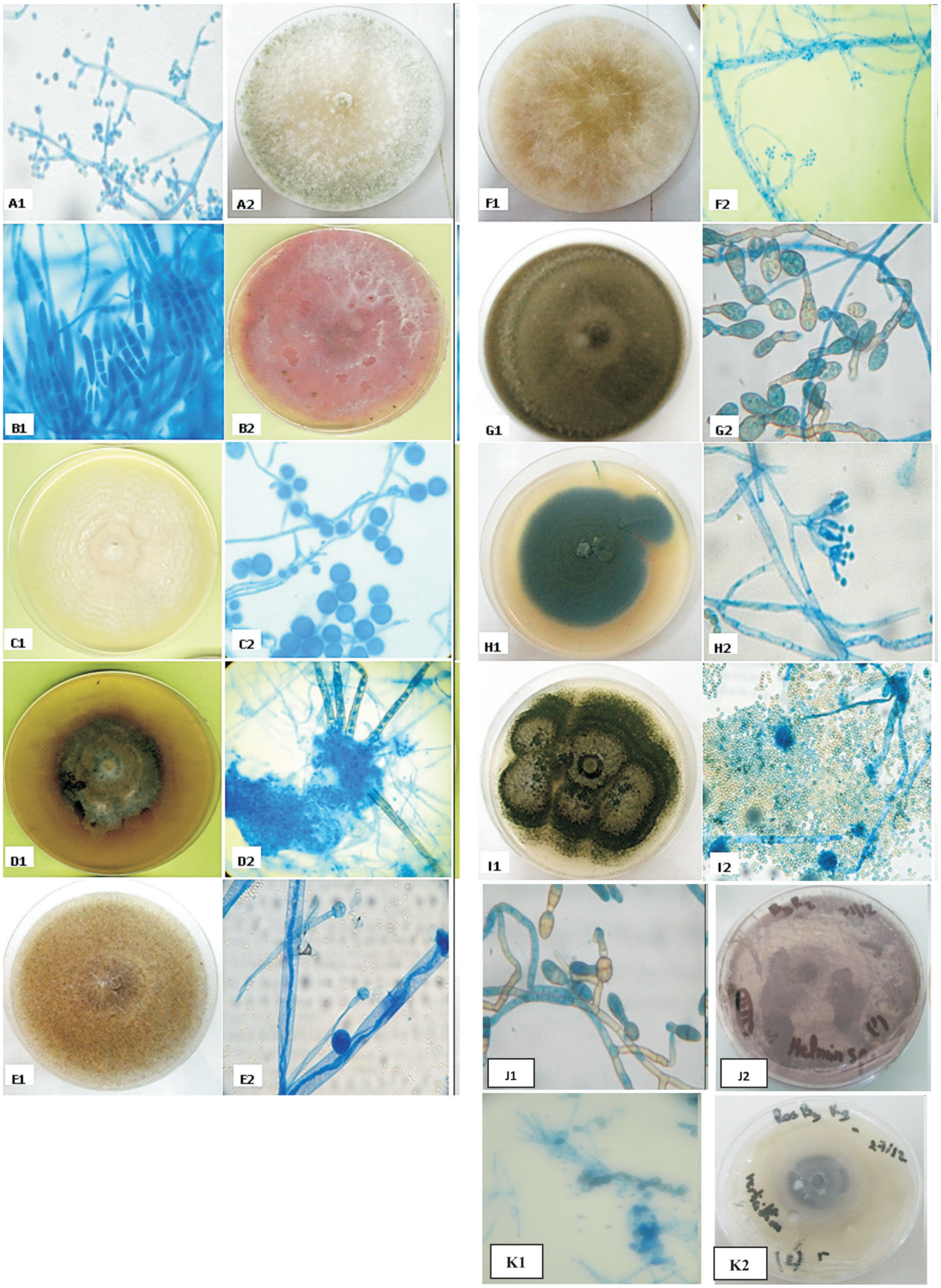

Fig. 10. Descriptive photos of isolated fungi. (1) Macroscopic aspects and (2) microscopic aspects; A: *Trichoderma*; B: *Fusarium*; C: *Pythium*; D: *Chaetomium*; E: *Mucor*; F: *Acremonium*; G: *Alternaria*; H: *Penicillium*; I: *Aspergillus*; J: *Helminthosporium*; K: *Verticillium*.

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