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STUDY OF MYCOFLORA ASSOCIATED WITH THE DECOMPOSITION OF SOLID GREEN HOUSEHOLD WASTE IN THE NATURAL ENVIRONMENT

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Abstract – Green household waste in general, banana and pomegranate wastes in particular, after their discharge into an aquatic or terrestrial ecosystem, were decomposed by the intervention of several biotic and abiotic factors. To our knowledge this kind of study has never been done before. For this reason we have set as our objective, the staggered in times characterization of mycoflora associated with the decomposition of two types of green household waste separately. During our work, the fungal microbial groups were isolated, purified and identified in MEA medium at a temperature of 28 °C, according to the known standards in environmental microbiology. Microbiological studies over times showed similar fungal densities associated with banana and pomegranate wastes in digestion in both media. In banana waste, microbial biodiversity has been more diverse. Among the species identified are *Aspergillus niger, A. flavus, A. terreus, Alternaria alternata, A. solani, Penicillium chrysogenum, P. cyclopium, P. digitatum* and other species. The fungal biodiversity associated with the decomposition of green household waste in natural environments can be exploited for agricultural, medical, and environmental interests.

INTRODUCTION

The quantity of waste in general and green household waste in particular in Morocco is increasing, especially pomegranate and banana wastes from its significant production of these two fruits, following demographic growth, changes in production and consumption patterns, and improvements in living standards (Mountadar *et al*., 2009; Hafidi, 2015; Awasthi *et al*., 2017 and El Barnossi *et al*., 2019). Almost all of that waste was landfilled or dumped directly into aquatic or terrestrial ecosystems (El Bada and Mountadar, 2012). Currently those wastes create serious health and environmental risks (Castaldi *et al*., 2008; Adam *et al*., 2009; Awasthi *et al*., 2014; Awasthi *et al*., 2015 and Gupta *et al*., 2015).

Several authors follow fungal microbial groups during the decomposition of organic matter in ecosystems including (Maamri, 1998; Iraqi, 2001; Agnolucci *et al*., 2013 and El Barnossi *et al*., 2018). However, so far systematic microbiological analyses of decomposing household waste remain insufficient.

Nowadays it appears more and more clearly that the studies of the sequences of microbial groups condition the understanding of the functioning of ecosystems. Whether it was for better management of natural resources that gives certain cases go unnoticed, or for taming species that can help solve agronomic, therapeutic and ecological problems. The study of fungal microbial sequences associated with decomposition of green household waste in an aquatic and terrestrial ecosystem was has poorly documented. For this reason, the objective of this work was to characterize the mycoflora associated with the degradation of green household waste (banana and pomegranate wastes) in water, and soil and to identify the different fungal isolates, of each degradation stage of these wastes.

MATERIALS AND METHODS

Biological Material

Two types of green household waste were used. Banana and pomegranate wastes that were collected from fruits juice sellers. These wastes in its fresh

state were put in place in buckets of 20 l. Fifty g of each waste were introduced into mesh bags, then introduced into buckets, half were filled with 15 l of spring water, and the other half were filled with 15 kg of garden soil of the Faculty of Sciences Dhar Mahraz, Fez, Morocco. Wastes digested in the soil they were irrigated every 5 days with 1 l of spring water throughout the study period to prevent desiccation of samples and maintain a humidity level close to saturation (El Barnossi *et al*., 2019).

Method for Isolation and Quantification of Fungi

The samples were taken every month of the study period (between February and May 2018) according to (Pitt and Hocking, 1997). The technique advocated by (Hachicha *et al.,* 1992 and Awasthi *et al*., 2017) was used for taking a representative sample. For each waste digested in water and soil. 10 g were removed and cut with a sterile chisel, then ground in a quantity of sterile spring water. The grinding was carried out with a rotating knife mill under aseptic conditions (El barnossi *et al.,* 2019). The suspension was then stirred for 2 hr in order to release the maximum of the microbial load (Gnonlonfin *et al*., 2013 and El Barnossi *et al*., 2018). The homogenate obtained corresponds exactly to the 10^{-1} dilution. Serial suspensions dilutions were then made from 10^{-1} to 10^{-8}, in the order of 1 mL in 9 ml of sterile distilled water. 0.1 mL in three replicates of each dilution and each sample, were separately deposited in the center of three Petri dishes of 90 mm diameter of modified MAE medium (7% waste extract). After plating, the dishes were incubated at 28 °C, in the dark for 7 days. The determination of the microbial load was done by colony counting and the results were expressed in fungal colony/g dry weight samples to be analyzed (Mouria *et al.,* 2012 and Guan *et al.,* 2018).

Purification of Fungal Isolates

Each different fungal colony was purified by depositing an inoculum of the apical part of the mycelium, in the center of the box in an inverted position, on the same isolation medium. The transplanted boxes were incubated at 28 °C. For each fungal colony, observations and photographs (when necessary) were taken daily until the entire box was colonized.

Identification of Fungal Isolates

To identify each fungal colony obtained. We based on macroscopic characteristics: growth, color, topography, and odor, and microscopic: the appearance of mycelial filaments (hyalinity, diameter, branching, etc.), the presence or not of anastomosis loops and partitions, special structures (cystide, cuticular cells, etc.), and fructification, were photographed, using a small photonic microscope of a camera, from fresh and dried smears, then colored with lactophenol blue solution (Bovio *et al.,* 2017) . To determine the genus of each fungal colony obtained, we adopted the Saccardo classification system (Barnette and Hunter, 1972 and Jedidi *et al*., 2018). Species identification was carried out by reference to different determination keys (Thom and Church, 1926; Gilman, 1957; Barnett, 1960; Ellis, 1971; Ellis, 1976; Domsch *et al.,* 1980; Nelson *et al.,* 1983; Wang and Zabel, 1992). The system adopted is that described by (Kirk *et al*., 2008).

STATISTICAL ANALYSIS

For each experiment, three repetitions were performed. Our results were designed and processed using the Graph Pad prism 5 software. The statistical analysis of the results obtained was done using the SPSS 20 software, according to a mean analysis (Student *t-test*) and an analysis of the variance (ANOVA I) at the threshold of $\alpha = 5\%$ (El Barnossi *et al*., 2019).

RESULTS AND DISCUSSION

Decomposition of Pomegranate and Banana Wastes in Water

Fungal Density

During the period of our study, the density of mycroflora associated with the degradation of banana and pomegranate waste in water evolved according to models that showed increases and decreases (Fig. 1). The highest density in banana waste (22.64×10^9 fungal colonies/g dw), and the highest density in pomegranate waste (19.95×10^9 fungal colonies/g dw) were obtained after 60 days of decomposition.

The counting of mycoflora as we have practiced it during our research, using the suspension dilution method, allows, despite the imperfections that can be blamed on it, to get a general idea of the importance of the fungi associated with our decomposing waste. These imperfections are due to the effect of grinding and homogenization, which according to many mycologists (Iraqi, 2001; Awasthi

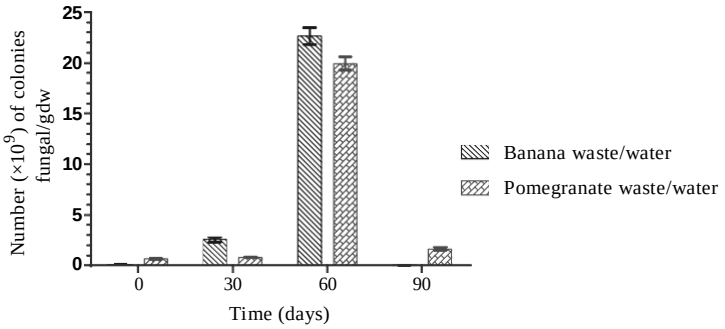


Fig. 1. Variation of the density of fungi associated with banana and pomegranate wastes in digestion in water.

et al., 2014 and Jedidi *et al.*, 2018) often result in the dilacerations of mycelia into partially altered and non-viable uneven fragments.

Mycoflora Associated with Banana and Pomegranate Wastes for Digestion in Water

The successive fungal groups associated with banana and pomegranate wastes digested in water during our study were gathered in (Table 2). The latter reveals that from the first day of the study to the end (between February and May 2018), banana and pomegranate wastes contain important sequences and successions of groups of fungi. The largest sequences were obtained in banana waste compared to pomegranate waste. These sequences and successions show qualitative and quantitative diversities that allow the identification during our study of 38 species belonging to different systematic groups. The highest number of species was obtained at the 60th day of decomposition of pomegranate and banana wastes in water. This variation was probably due to biotic and abiotic factors associated with decomposing waste (Iraqi, 2001). The study by Anastasi *et al*., (2004) shows the total mycoflora was composed of 48 mitosporic genera, 17 ascomycete genera and 4 zygomycete genera. Moreover, 14 SM morphotypes were collected in addition to several basidiomycetes. In any case, our results show that the number of fungal colonies varies with time, the decomposition medium and the nature of the substrate studied. This variability is a common event in microbiological studies of natural environments (Awasthi *et al*., 2014 and El Barnossi *et al*., 2018). It is unequivocally the result of the effect of biotic and abiotic factors. In general, the qualitative and quantitative differences noted between our results and those in the literature lead us to believe that a given microflora ecological group corresponds to a given plant substrate (banana and pomegranate wastes). This concept was confirmed by several authors, including Awasthi *et al.*, (2017) show that *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* were identified as universal saprophytic fungi on various kind of organic and food wastes.

For fungal successions, a decomposer can replace other species when changes in the substrate have interacted with changes in saprophytic competitiveness and inoculation potential, giving it a decisive advantage (Vilanova and Porcar, 2016 and Jedidi *et al*., 2018). Thus, the microorganisms develop particular ecological strategies.

In our opinion, the successions of fungal waves obtained during our study constitute a model capable, on the one hand, of providing important information on the biology of decomposing fungus populations and, on the other hand, of allowing the study of the relationships between colonizing capacity and competitiveness.

Decomposition of Pomegranate and Banana Wastes in the Soil

Fungal Density

The results obtained during our work (Fig. 2) show that the density of mycroflora associated with the decomposition of banana and pomegranate wastes in the soil increases with time. However, the density

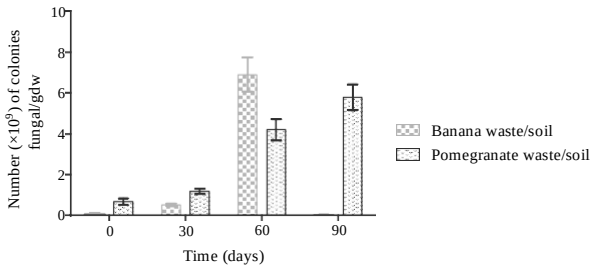


Fig. 2. Variation of the density of fungi associated with banana and pomegranate wastes in digestion in the soil.

Table 1. Macro and microscopic characteristics of some fungal isolates associated with decomposed waste in the water.

a. Pictures in front of some fungal isolates; b. Pictures in back of some fungal isolates; c and d. Microscopic aspects (mycelial filaments, diameter, branching, spore and conidiophore, etc.) of some fungal isolates.

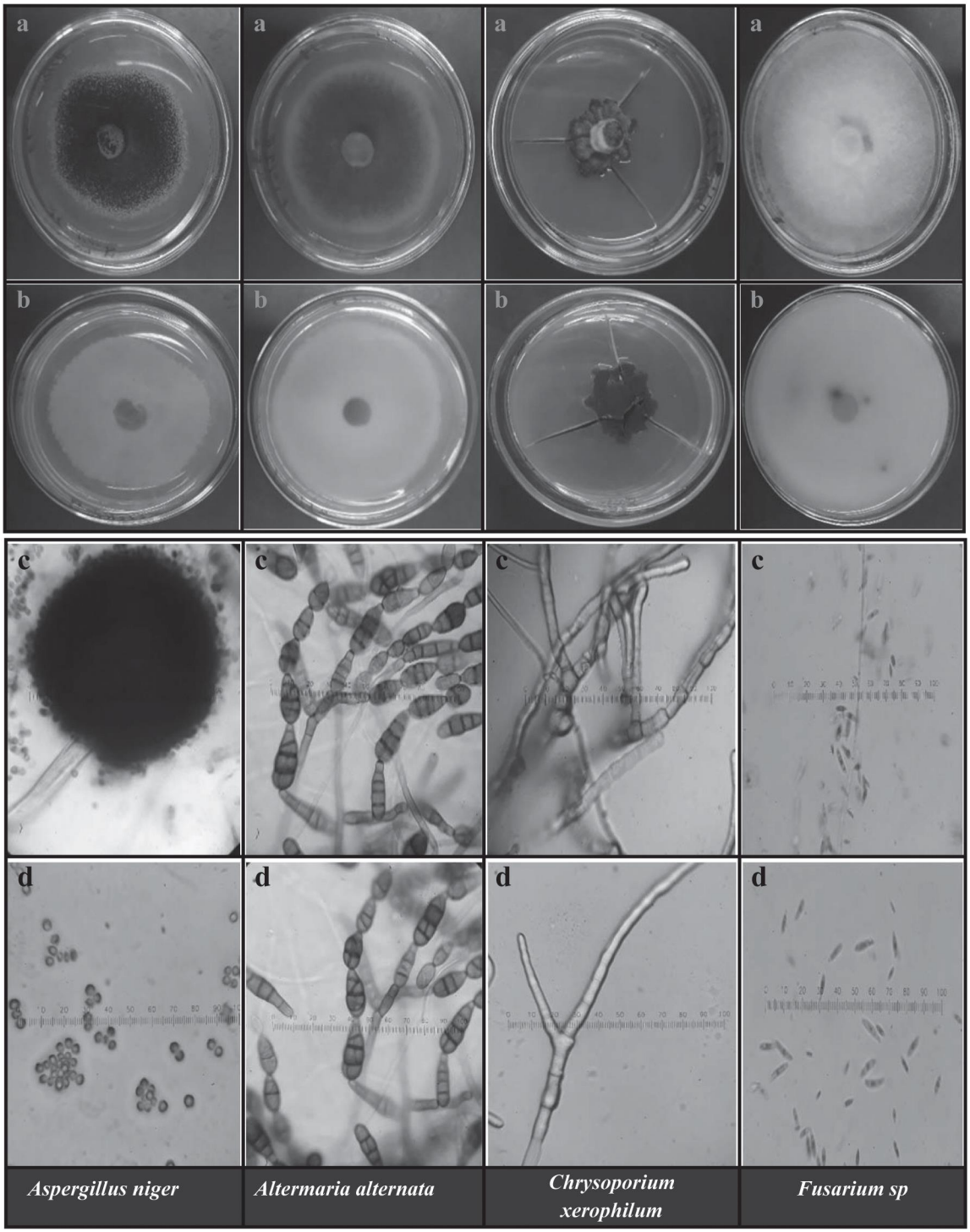


Table 2. Fungal sequences associated with decomposing banana and pomegranate wastes in water.

Waste Time	Pomegranate waste digested in water	Banana waste digested in water
0 days	*Fusarium oxysporum; Penicillium sp; P. glabrum; P. islandicum*	*Aspergillus flavus; Aspergillus niger; Aspergillus ochraceus; P. cyclopium; P.islandicum; P. simplicissium*
30 days	*Cladosporium cladosporioides; P.funiculosum; P. citreonigrum; P.citrinum; P. simplicissium*	*Alternaria solani; Mucor; P. expansum; P. fellutanum; P. funiculosum; P. islandicum; P. variabile*
60 days	*Alternaria alternata; Aspergillus flavipes; Aspergillus phoenicis; Chrysoporium xerophilum; P. chrysogenum; P. islandicum; P. janthinnelum*	*Aspergillus fumigatus; Aspergillus japonicas; Cladosporium herbarum; Chrysoporium xerphilum; P.cyclopium; P.expansum; P.islandicum; Ulocladium atrum*
90 days	*Moniliella acetoabutens; P. citreonigrum; P. expansum; P. islandicum*	*Aspergillus terreus; Fusarium moniliforme; Drechslera dermatioidea; Moniliella acetoabutens; P.thomii*

of the mycoflora associated with banana waste shows a remarkable decrease, after 90 days of degradation. The highest density in banana waste (6.9×10^9 fungal colonies/g dw) obtained after 60 days of decomposition. While the highest density in pomegranate waste, (5.78×10^9 fungal colonies/g dw) obtained after 90 days of decomposition.

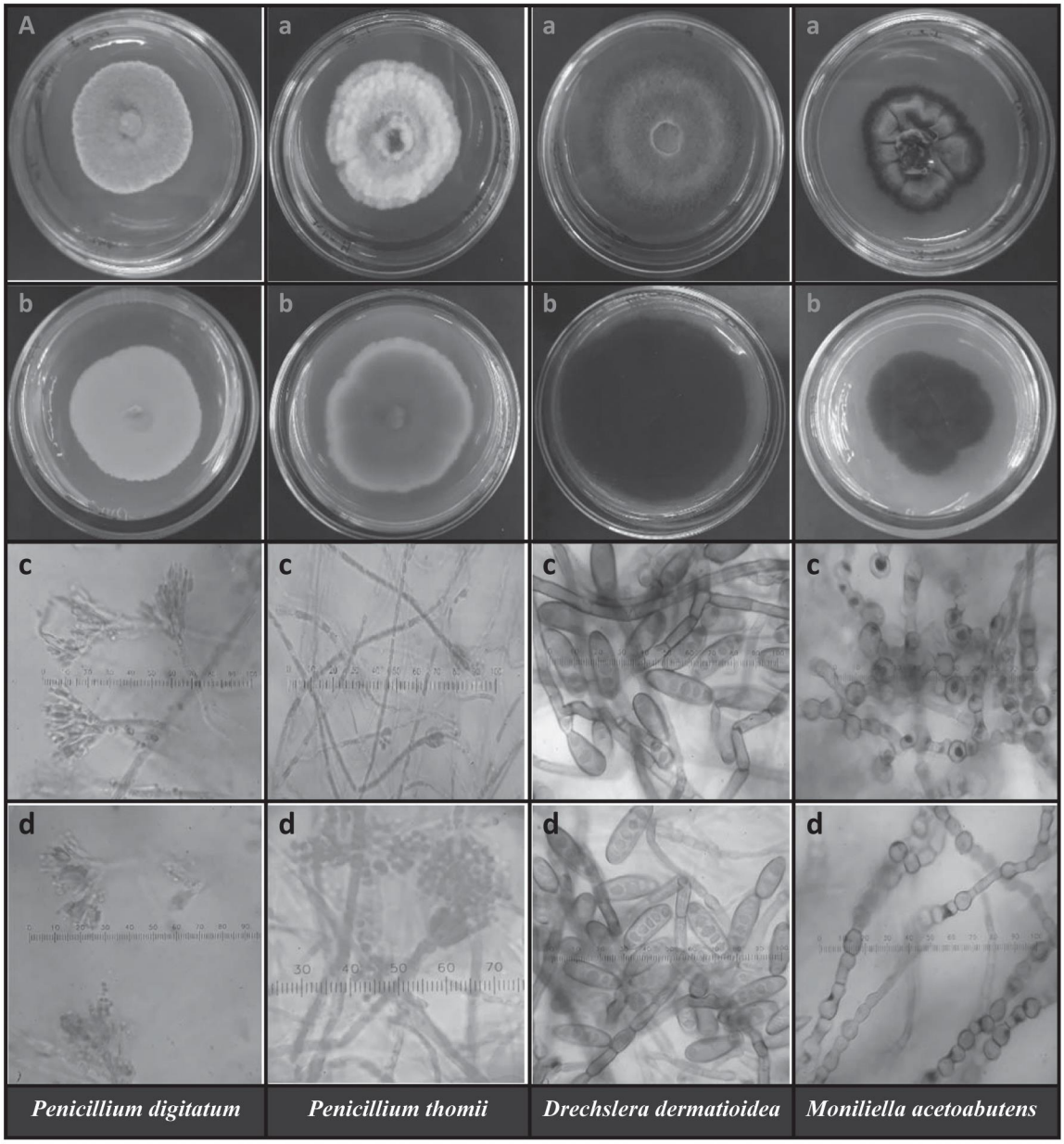
Mycoflora Associated with Banana and Pomegranate Wastes in Digestion in Soil

The fungal sequences associated with decomposing banana and pomegranate wastes in the soil were collected in (Table 3). The results obtained show that banana and pomegranate wastes contains sequences of true fungi depending on the substrates studied and the decomposition medium. The largest sequences were obtained in banana waste compared to pomegranate waste and in water degradation compared to soil. These sequences and successions show important qualitative diversities (Table 4) which allow the identification during our study of 30 species (Table 3) belonging to different systematic groups. The largest number of species was obtained at 60 days in the digested banana waste. While in the pomegranate waste was obtained at the 90th day of degradation. The population of mycoflora observed during our study was consistent with those reported by (Larbi, 2006) which has shown that some composts contain approximately 10^6 CFU/g dw of fresh compost from fungal population, and by (Riachi, 1998) which has shown that in green waste compost the fungal load fluctuates between 10^5 and 10^6 CFU/g dw. Anastasi *et al.*, (2005) also reported fungal load values fluctuating between 5×10^4 and 8.2×10^5 CFU/g dw in a compost and between 5.3×10^4 and 4×10^5 CFU/g dw in a vermicompost. They isolated thirteen species of fungi; *Aspergillus fumigatus, A. flavus, A. ochraceus, Aureobasidium pullulans, Chaetomium globosum, Cladosporium cladosporioïdes, C. harbarum, Emericella nidulans,*

Table 3. Fungal sequences associated with decomposing banana and pomegranate wastes in the soil.

Waste Time	Pomegranate waste digested in soil	Banana waste digested in soil
0 days	*Fusarium oxysporum; Penicillium sp; P.glabrum; P. islandicum*	*Aspergillus flavus; Aspergillus niger; Aspergillus ochraceus; P. cyclopium; P. islandicum; P. simplicissium*
30 days	*Acremonium charticola; Fusarium sp; P.citreonigrum*	*Aspergillus phoenicis; Paecilomyces variotii; P. chrysogenum; P. digitatum; P. glabrum*
60 days	*Moniliella acetoabutens; Moniliella suaveolens; P. chrysogenum; P. citreonigrum; P. glabrum; P. simplicissium; P. variabile*	*Acremonium charticola; Fusarium solani; Fusarium sp; Drechslera dematiodea; P. chrysogenum; P. expansum; P. islandicum; P. rugulosum*
90 days	*Cladosporium cladosporioides; P. digitatum; P. glabrum; P. thomii; P. islandicum*	*Alternaria alternata; Fusarium sp; P.citreonigum; P. funiculosum; P. glabrum*

Table 4. Macro and microscopic characteristics of some fungal isolates associated with decomposed waste in the soil. a. Pictures in front of some fungal isolates; b. Pictures in back of some fungal isolates; c and d. Microscopic aspects (mycelial filaments, diameter, branching, spore and conidiophore, etc.) of some fungal isolates.



Humicola grisea, Penicillium simplicissimum, Scopulariopsis brumptii, Scytalidium lgnicola and *Trichoderma harzianum*. Other studies have only isolated solitary species such as *Fusarium sp. Scopulariopsis brevicaulis* (Ryckeboer *et al*., 2003) from different composts or decomposing waste. In our opinion, the successions of fungal waves obtained during our study constitute a model capable, on the one hand, of providing important information on the biology of decomposing fungus populations and, on the other hand, of allowing the study of the relationships between colonizing capacity and competitiveness.

CONCLUSION

The study of fungal successions as we have done, and the results that have resulted, lead us to believe in the property of banana and pomegranate wastes in digestion in water and soil to attract a varied

mycoflora. This mycoflora must be taken into account in greater depth, to define the specificity of each strain and explain the decomposition and microbial succession phenomena, and to isolate by selective screening fungal species that may be used in pharmacological, phytopathological and industrial applications.

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