ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Bioremediation of pesticide (mancozeb) by two *aspergillus* species isolated from surface water contaminated by pesticides

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ABSTRACT

Pesticides are a means of control, the most effective against major diseases of crop plants, and which are necessary in maintaining or increasing agricultural yields. However, most of these molecules are highly toxic, persistent and slowly biodegradable and constitutes a real danger to the environment and human health.

Algeria is ranked among the countries that use larger amounts of pesticides. So, about 400 pesticides are registered in Algeria. This situation is particularly worrying especially since use of pesticides should be repeated periodically. So, in this context, we conducted our study which consists to verify the catabolic metabolic capabilities of two fungal strains (*Aspergillus niger* and *Aspergillus flavus*). To do this, the influence of fungicide mancozeb on strains growth was determined. The biodegradation ability of two microbial species toward the fungicide was also evaluated. The tolerance of these two strains towards this fungicide were evaluated in vitro from a set of biochemical criteria after incubation at 30°C in the presence of the pesticide as sole source of carbon and energy. Mancozeb biodegradation rates are measured by gas chromatography (GC).

The biodegradation tests show that mancozeb disappearance rates are variable depending of the fungal strain. The *Aspergillus flavus* catabolic power seems better with removal rate exceeding 50%. This strain appears interesting and therefore, it could be envisaged in the bioremediation process.

KEY WORDS: Pesticide, mancozeb, *Aspergillus niger*, *Aspergillus flavus*, bioremediation, surface water.

1. INTRODUCTION

Due to their ecotoxicity, their potential for bioaccumulation, and their endocrine action, these molecules represent a risk to the environment due to their lack of selectivity in relation to their targets, and their potential distribution in d other environmental compartments (Barriuso, 1996; Irace-Guigand, 2004; Pesce, 2008; Calvet, 2005).

Bioremediation is a set of biological technique to remove environmental pollutants. These techniques can, using the capacity of certain organisms and microorganisms to degrade organic and / or eliminate the soil or water the polluting substances. Biodegradation is one of them: this involves the use of microbial strains (bacteria and / or fungi) to clean the soil and water.

It is in this context that fits our work is to study the biodegradation of Mancozeb (pesticide widely used against fungal diseases of crops in the North-east of Algeria) by two species of *Aspergillus*.

2. MATERIAL AND METHODS

The microbial strains: The microbiological material consists of two fungal species *Aspergillus niger* and *Aspergillus flavus*. They were isolated from water polluted by pesticides in agricultural use to a localized region in the north-east part of Algeria (ben M'Hidi).

Aspergillus niger is an important species in economic terms is a filamentous fungus ascomycete the order of Eurotiales. This fungus is a ubiquitous contaminant that is usually harmless.

Aspergillus flavus is a species of fungus ascomycete. This fungus is very cosmopolitan. *Aspergillus flavus* is the main producer of aflatoxins.

Pesticide: Mancozeb is a systemic fungicide belonging to the family Dithiocarbamate. Mancozeb fungicide belongs to the group commonly called ethylenebis (dithiocarbamate) (EBDC), it decomposes into ethylene thiourea (ETU), the cumulative risk profile is also considered.

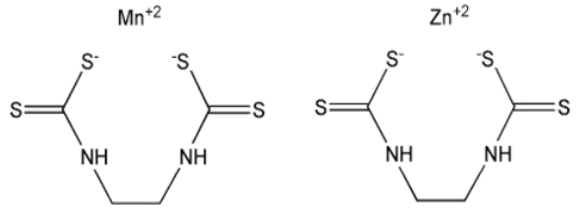

Fig.1. Chemical structure of mancozeb

Evaluation of the biodegradation of the pesticide mancozeb: The cultures were carried out in batch mode in Erlenmeyer flasks containing 100 ml of GS medium supplemented with glucose stirred on an orbital plate 180 rpm for 48 hours. The fungicide solution prepared in DMSO / ethanol (50/50, V / V), sterilized by filtration through a Millipore membrane 0.22 µm is added to cultures of aseptically to a final concentration of 100 mg /l. The abiotic degradation (culture medium + substrate without inoculum) are included in the trials. Each series of experiments was

made in triplicate. Every 24 hours, there was sampling to verify strains growth by measuring the optical density at a wavelength of 660 nm and the concentration of oxygen in the medium. Growth of fungal strains was confirmed by evaluation of the dry weight after 5 days of incubation. The molecules biodegradation rates are calculated from samples (1 ml) fulfilled at T0J and T5J time after adding the pesticide. These samples were filtered through Millipore membrane 0.45µm and injected directly without extraction. The evaluation of residual pesticides is made by gas chromatography GC.

3. RESULTS AND DISCUSSION

Table.1. Biochemical parameters of cultures of *Aspergillus niger*

Time in hours	pH	Optical density	Oxygen rate (COD in mg/l O_2)
24h	3.7	0.39	0.1
48h	3.4	0.42	/
72h	2.8	0.56	/
96h	3.6	0.59	/
120h	3.1	0.72	0.092

Table.2. Biochemical parameters of cultures of *Aspergillus flavus*

Time in hours	pH	Optical density	Oxygen rate (COD in mg/l d'O_2)
24h	5.4	0.56	0.079
48h	4.8	0.66	/
72h	4.5	1.16	/
96h	4.6	1.40	/
120h	4.1	1.81	0.054

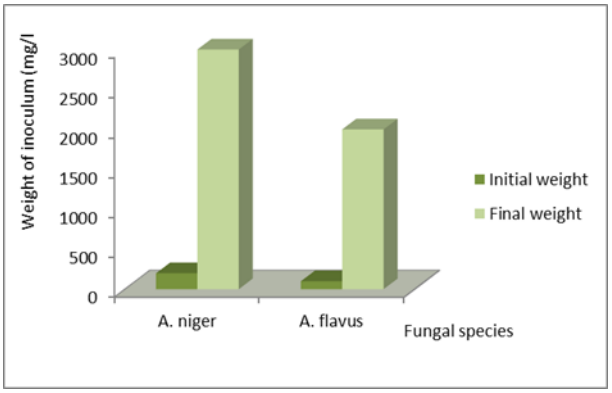

Fig.2. Dry weight of *Aspergillus niger* and *Aspergillus flavus* inoculum

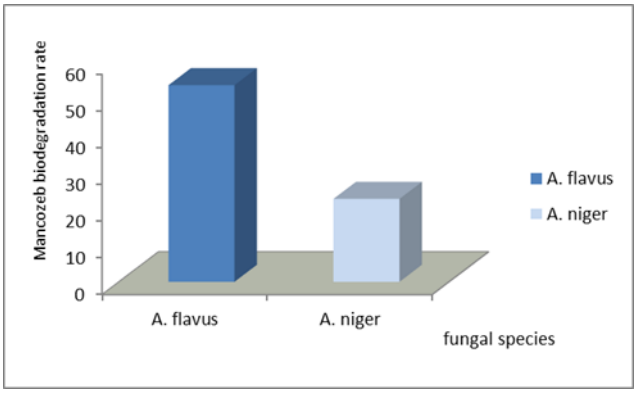

Fig.3. Histogam of Mancozeb biodegradation rate by *Aspergillus niger* and *Aspergillus flavus*

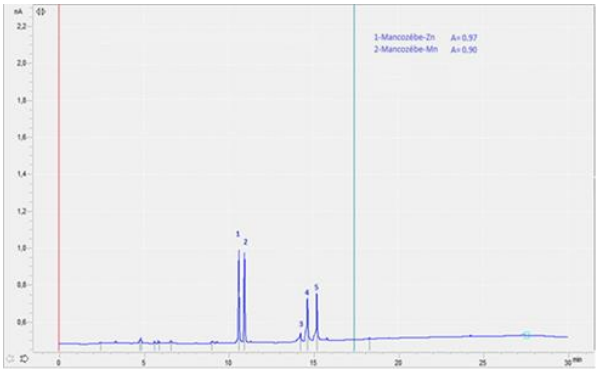

Fig.4. CG chromatogramme of Mancozeb biodegradation rate by *Aspergillus niger*

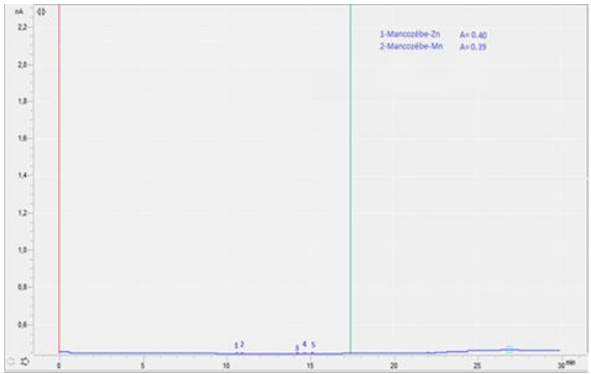

Fig.5. CG chromatogramme of Mancozeb biodegradation rate by *Aspergillus flavus*

After five days of incubation in the presence of pesticides, the dry weight of the biomass of both strains is significantly higher compared to the initial weight. The results obtained show an increase in the inoculum and acidification of the environment with a perfect correlation between the increase in dry weight and decrease in pH. These results are in perfect concordance with those obtained by Gillian and Turbur (2001).

Metabolic capacity varies with the fungal strain and the molecules used as the carbon source or nitrogen) during the growth phase (Bordjiba, 2001). And strains of *Aspergillus* and Penicillium incubated with metobromuron,

metribuzin give different answers. According to Keith and Telliard (1979), phthalates, dimethyl terephthalate is converted by *Aspergillus niger* with a significant rate. However, the same molecule of dimethyl terephthalate is converted by Sclerotium rolfsii but the degradation is only partial (Sivamurthy, 1991).

In our study, a good response from the strain of *Aspergillus flavus* with strong power degradation was recorded against the mancozeb at a rate exceeding 50%. However *Aspergillus niger* has caused the disappearance of only 22.59% of the mancozeb initial rate. These results are quite similar with those reported by Bordjiba (2003), with the same species in the presence of metobromuron and metribuzin.

Aspergillus niger and *Aspergillus flavus* are species resistant to various pollutants in soil and water with high concentrations of herbicides and fungicides (Domsch, 1980; Pitt, 1979). These are very common saprophyte mushrooms in the world, reported in several warm regions, tropical regions and those with temperate climate. They were isolated on various substrates, at different depths of the soil (Steiman, 1992; 1997; Guiraud, 1995).

Both tested strains are able to grow well in the culture medium in the presence of pesticides, they are able to survive and adapt in difficult environmental conditions (polluted areas). For it, both fungi *Aspergillus niger* and *Aspergillus flavus* are capable of biodegradation. However, the species *Aspergillus flavus* has a higher capacity than *Aspergillus niger*.

4. CONCLUSION

This study aims to determine the capabilities of the two *Aspergillus* strains to degrade mancozeb fungicide molecule studied. The major element that emerges from this work is that the strain of *Aspergillus niger* is better, it has interesting metabolic capabilities probably inducing enzymes involved in biotransformation reactions. Therefore it could be considered in the bioremediation process. However, to consider its use, it would be interesting to test it on a wide range of compounds to determine the extent of its tolerance and its catabolic abilities.

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