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Biodegradation of three fungicides by Pseudomonas cepacia isolated from a soil in the north-eastern Algeria

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Abstract

Generally the molecules of pesticides in soils, rainfall and surface water are dangerous for the environment. Most of them are classified by the EU as priority pollutants. And the risk to the environment has led to a keen interest vis-à-vis bioremediation technologies.
Many pesticides are aromatic compounds that are derived from benzene or neighboring hydrocarbon rings. The opening of the benzene ring is a chemically difficult reaction that requires powerful reagents, it is striking that, conversely, and it is easily accomplished by microorganisms. That is why the use of microorganisms for biotransformation of environmental pollutants has become a concern of many researchers.

Therefore, our objective is to investigate the effect of three molecules of pesticides belonging to the family of benzimidazoles and imidazoles on the soil parameters and their biodegradation by bacterial microflora isolated from contaminated sites.

To do this, the soil physico - chemical and biological parameters were determined. Analyzes have focused on pH, humidity, nitrates, electrical conductivity, organic matter and bacterial microflora. The physico-chemical parameters were analyzed by standard methods according to the general guidelines for storage and handling. The identification of isolated and purified bacteria was performed by identification galleries API 20 E / NE. Biodegradation tests of three molecules of pesticides were conducted in batch culture in GS medium. After 5 days, the rates of biodegradation were evaluated by GPC.

The results show that there is soil pollution. The values of some parameters are below the prescribed standards, particularly organic matter and electrical conductivity. The removal rate of pesticide exceeds 50% for Benomyl and it is depending of the nature of the chemical studied.

Keywords: biodegradation, carbendazim, benomyl, fenapanil, soil, Pseudomonas cepacia.

INTRODUCTION

The development of the use of synthetic organic products for combating fungi, weeds and plant parasitic nematodes date more than fifty years after the discovery of the insecticide dichlorophenyl - trichloroethane (DDT) and the herbicide 2,4-dichloro-phenoxyacetic acid 2,4 - D. By this time it was observed that 2, 4 - D persisted more in sterilized soil in a witness suggesting microbial action in the soil disposal ground. This powerful herbicide is indeed degraded by many

microorganisms. Instead DDT was found to be particularly stable and resistant to biodegradation and posed serious problems of environmental pollution.

Many pesticides are aromatic compounds that are derived from benzene or neighboring hydrocarbon rings. The opening of the benzene ring is a chemically difficult reaction that requires powerful reagents, it is striking that, conversely, it is easily accomplished by microorganisms. That is why the use of microorganisms for biotransformation of environmental pollutants has become a concern of many researchers.

Most work to date dealing with the biodegradation of pesticides by the total biomass of microorganisms [13] very few are those related to fungal microflora. Therefore, our objective is to investigate the effect of a three molecules of pesticide on the soil parameters and their biodegradation by microflora isolated from contaminated sites

II. MATERIEL AND METHODS

A. Soil samples

Samples are taken from areas farming located in the north-eastern part of Algeria intended to the vegetable crops and subject to the effects of many pesticides for a long time.

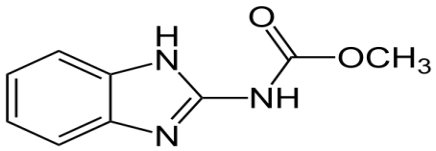
B. Bacterium

The bacterium was selected from the total microflora isolated of analyzes soil samples. After identification, the strain was maintained on LPGA medium and stored at 4 ° C. from

C. Chemicals

The molecules studied are fungicides. Two molecules belonging to the family of benzimidazoles (benomyl and carbendazim) and one molecule belonging to imidazoles (fénapanil).

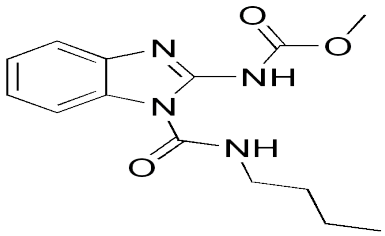
Carbendazim is a systemic fungicide with protective and curative action. It was absorbed through the roots and green tissues, with translocation acropetally. Acts by inhibiting the development of germ tubes, the formation of appressoria, and the growth of mycelia.



$C_9H_9N_3O_2$

Fig. 1: formula and chemical structure of carbendazim

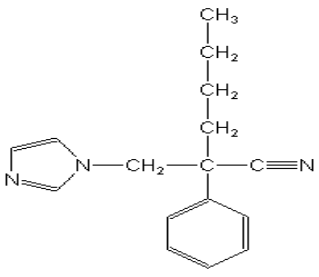
Benomyl is a systemic fungicide. It is belonging to the family of benzimidazole pesticides that is selectively toxic to microorganisms and invertebrates, especially earthworms. Benomyl binds to microtubules, interfering with cell functions,: such as meiosis and intracellular transportation. The selective toxicity of benomyl as a fungicide is possibly due to Heightened its effect on fungal Rather than mammalian microtubules [1]



$C_{14}H_{18}N_4O_3$

Fig. 2: formula and chemical structure of benomyl

The fenapanil is a systemic fungicide; it acts on specific phenomena of biosynthesis. It causes the inhibition of enzymes involved in the synthesis of sterols, resulting in disruption of the functioning and the formation of cell membranes of microorganisms.



$C_{16}H_{19}N_3$

Fig. 3: formula and chemical structure of fenapanil

D. Analysis of physico-chemicals parameters.

The analyzes are focused on pH, organic matter, carbon, electrical conductivity, and limestone. These parameters were evaluated by standard methods [3] and compared with the scales reported in Durand [6]

E. Evaluation of biological parameters: total soil microflora

The soil suspension is prepared by the dilution method Described in the standard DIN 54379 for the enumeration of colonies.1 g of soil from each sample is stirred in 100 ml of sterile Ringer's solution [5].
The resulting suspension is diluted. The dishes are incubated at 30 °C temperature for 5 to 7 days. The isolation of species is made on gelosed medium. The petri dishes are made in triplicate for each case and were incubated at 30°C.

F. Biodegradation tests

The cultures are carried out in synthetic medium and Galzy Slonimski (GS) [9], supplemented with glucose (5 g / l) at pH 4.5. The inoculum is then added to the culture medium. Incubation is carried out at a temperature 30 ° C under illumination of 1200Lux (12H/24H). The cultures are stirred on orbital plate at speed of 180 rev / min for two days. Incubation is performed at 30 ° C. The pesticides suspension are prepared in DMSO /ethanol (50/50, V / V), sterilized by filtration through a membrane Millipores of 0.2 µ then added to the cultures in an aseptic manner to a final concentration of 100 mg / l. The witnesses of abiotic degradation (middle + substrate without bacteria) are included in the trials. Each series of experiments is made in triplicate. Samples (1 ml) are performed at time t_0 and 5days after the addition of pesticide. These samples are filtered through a Millipore membrane (0.45 µm) and injected directly without extraction (three injections for each sample.

G. Evaluation of the biodegradation of three molecules: benomyl, carbendazim, Fenapanil

The evaluation of the disappearance of the product is done by gas chromatography (GPG) of the residual rate. This amount subsequently, determines the percentage removal which is converted to mg of product per g of transformed dry weight of inoculum.

III. RESULTS AND DISCUSSION

A. Soil parameters

Soil parameters	
pH	7,8
Carbone (%)	6,76
Limestone (%)	19,25
Organic matter (%)	11,05
Electrical conductivity (mS/cm)	0,12
Total microflora (Colonies/g soil)	51407

Table 1: Soil paramaters

The values of different parameters determined are compared with others scales. They indicate that we are in the presence of an alkaline soil with low percentage in organic matter and very low electrical conductivity probably due to the presence of pesticides [3].
The total bacterial microflora isolated is consisted of 51407colonies / g soil. The Isolated bacterial species are probably acclimated to the presence of pesticides used in the soil they do not vary from one sample to another; we find the same genera and same species in almost all samples with variable frequencies. The difficult environmental conditions caused a loss of diversity of the microflora [2]. Among The species most frequent in all soil samples is *Pseudomonas cepacia.*

B. Biodegradation of benomyl, carbendazim, Fenapanil

This study reveals the potential of Pseudomonas strain in the transformation / biodegradation of three molecules: benomyl, carbendazim and fénapanil and metabolic capabilities vis-à-vis each type of product used as a source of carbon and energy

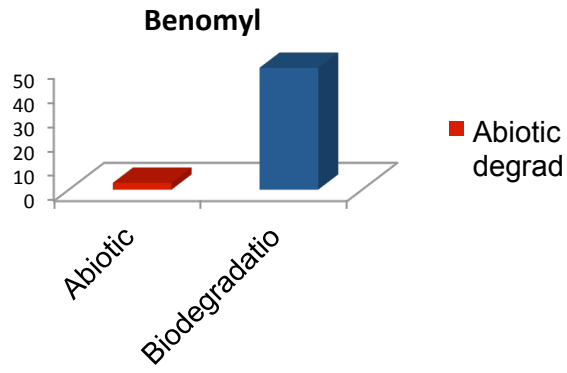


Fig 4: Percentage of Biodegradation of Benomyl by Pseudomonas cepacia

The tested strain appears to be effective on the benomyl since the degradation rate is 50%. Further work carried out on a strain of Pseudomonas have signaled that the bacterium can use the benomyl as the sole carbon source for growth and arrives to hydrolyze the side chain forming the butylcarbamoyl carbendazim (MBC). The formed N-butylcarbamate is in turn hydrolyzed to n-butylamine. The product so formed is then converted to 2-aminobenzimidazole (AB-2) [8]

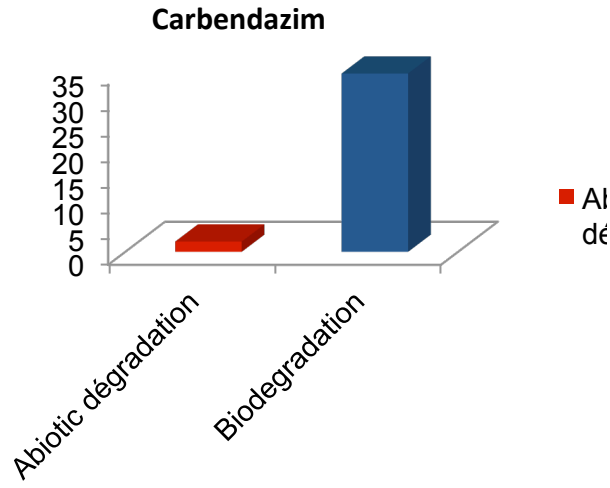


Fig 5: Percentage of Biodegradation of carbendazim by Pseudomonas cepacia

In terms of carbendazim, the degradation rate is lower than that recorded for benomyl. Only 34,62% of the initial amount are transformed. However, different results are obtained from a study on one specie o f Pseudomonas (Pseudomonas sp) isolated from soil that has been able to use carbendazim as sole source of carbon and energy and has degraded the rates of 87.1 and 99.1% after three days of incubation at pH 7 and at elevated temperature. By comparison with these results, our Pseudomonas cepacia strain could potentially be useful after optimization of culture conditions such as pH, temperature and incubation time. The actinomycete Nocardioides sp. SG-4G is capable of use in the metabolizing carbendazim to 2-hydroxybenzimidazole which in turn could be used as source of carbon and energy for growth [12].

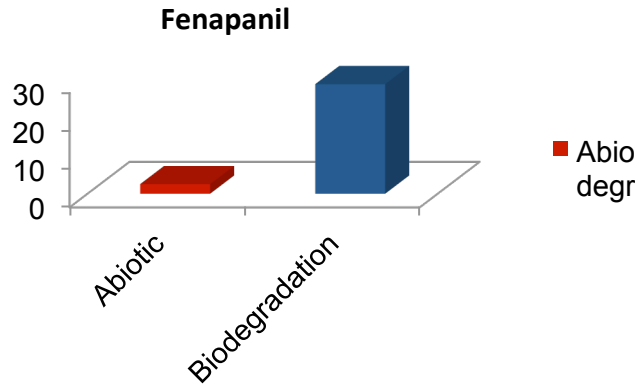


Fig 6: Percentage of Biodegradation of fenapanil by Pseudomonas cepacia

The fénapanil is weakly metabolized in the presence of this strain of pseudomonas (28,78) ; it is one of the most recalcitrant compounds degraded with difficulty [7]. In our opinion, this could be due to the presence of two benzene cyclic rings in its chemical structure and we know that the opening of the benzene ring is a difficult chemically reaction. In addition, the presence of a bond triple and several butyl substituents seems to have an effect on biodegradation and can increase the resistance to decomposition [4]. This highlights the importance of the role played by the nature of a substance in chemical biotransformation ([14], [10], [11]).In sum, the profiles of the GC show the presence of metabolites in extracts of culture medium tested (fig 1, peak). This indicates that the loss of the fungicide is due to its biotransformation.

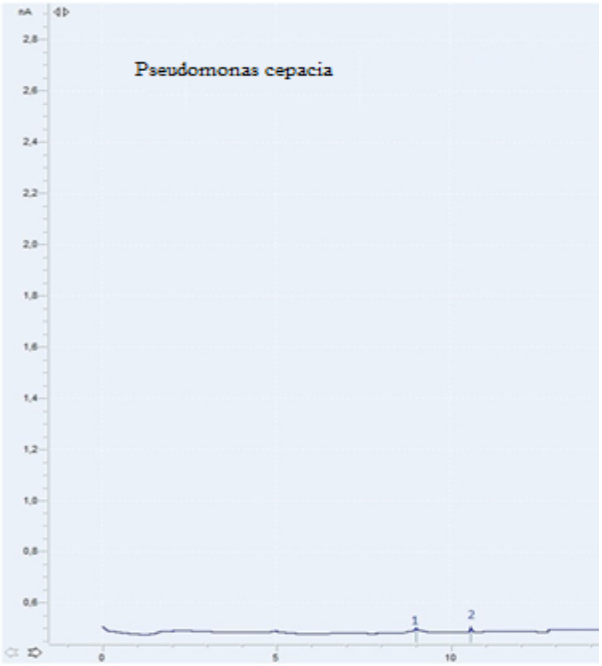


Fig. 7: chromatogramme GPC biodegradation of three pesticide molecules: carbendazim, benomyl, fenapanil by *Pseudomonas cepacia*

IV. CONCLUSION

Already, we can deduce that Benomyl is the compound more easily degraded by this strain of Pseudomonas. Conversely, the fénapanil is the most resistant to biodegradation. The polluted environment has probably favored the selection of strains expressing specific enzyme systems to certain substances but less effective, unable to degrade a large number of molecules of different chemical nature.

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