Degradation of ziram fungicide using organisms isolated from rotten grapes and soil

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Abstract

Samples were collected from various areas in Tamil Nadu and Maharashtra analyzed for ziram degradation. Soil samples and grapes were cultured on minimal media containing 1% ziram fungicide degradation. The isolates were identified and degradation of Ziram at different pH, concentration, temperature, static and non static condition. The HPLC analysis along with spectrophotometry was used to confirm degradation.

Keywords: Ziram, transformation, fungicide, degradation.

INTRODUCTION

The most intelligent species on earth poisons its food before eating it; reveal the stark reality of destruction that we face today due to the relentless and injudicious use of pesticides. We are continuously fed on pesticide diet as they reach our food, drinking water, air, etc. Fungicides are biocidal chemical compounds or biological organisms used to kill parasitic fungi or their spores. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections. Chemicals used to control oomycetes, which are not fungi, are also referred to as fungicides, as oomycetes use the same mechanisms as fungi to infect plants. Fungicide residues have been found on food for human consumption, mostly from post-harvest treatments. Some fungicides are dangerous to human health, such as vinclozolin, which has now been removed from use. Ziram is also a fungicide that is thought to be toxic to humans if exposed so chronically. A number of fungicides are also used in human health care. MARC BOURGIN., JOE L ALBET AND FRE DE RICVIOLLEAU (2013).


MATERIALS AND METHODS

Sample collection

Three samples were used in this process. Sample I are the grapes which were rotten, Sample II is the soil collected from the grape farm in Nasik and the sample III is the grapes in which the fungicide ziram was added and they were raw grapes from Nasik.

Isolation and identification of organisms

Primary screening was done to isolate the microorganisms that are able to tolerate ziram fungicide and use it as a source of energy. For this purpose minimal medium was used. Minimal agar with 1% of ziram was used for isolation. The isolates were identified by staining and Biochemical analysis (Willey et. al., 2008).
Table 1. Biochemical tests

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase test</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Catalase test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Citrate utilization test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Methyl red test</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>Vogesproskuer test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Triple sugar ion test</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Coagulase test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Gelatine test</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Durai r., Ng P.C., Hoque H. Methicillin-resistant staphylococcus aureus: an update. AORN J. 2010;91(5);599-606 (Pubmed).**

**Effect of pH, Concentration, Temperature, static and non-static condition**

The effect of pH, Temperature, Concentration of fungicide for optimal growth of the bacterial isolates were determined. Different parameters like temperature (5°C, 15°C, 37°C, 45°C), pH (3, 5, 7, 9) and concentration (1% and 2%) were determined. The absorbance was measured at 690nm in calorimeter.

**Transformation**

The plasmids were isolated from bacterial strains and they were transformed into host culture *E.coli*DH5α strain was used as the host culture. For isolation of plasmid
DNA alkaline lysis method was used. Competent cells were prepared by using 0.1% calcium chloride solution. Blue white screening was used for checking transformation which involved the use of gentamycin, erythromycin, X-Gal and IPTG.

**High performance liquid chromatography**

A standard was prepared by dissolving the fungicide in ethanol ziram was detected at 590nm, the best samples were given to HPLC analysis.

**RESULTS AND DISCUSSION**

**Isolation of microorganisms**

For isolation of organisms, minimal media was used to show high growth of organisms compared to nutrient medium. The minimal medium was supplemented with ziram and growth was observed with 24-48 hours. Four organisms were isolated which were able to degrade ziram fungicide. HAQUE M.M. AND MUNEER M. 2005. ‘Photo catalysed degradation of a fungicide, thiram in aqueous suspension of titanium dioxide’. Indian Journal of Chemistry technology. Vol. 12, pp. 68-72.

**Staining and Biochemical analysis**

The bacteria isolated were identified using Gram’s staining method. And fungi by Lactophenol cotton blue staining

**Effect of pH, Concentration, Temperature, static and non-static condition**

**Concentration**

The OD (Optical Density) values for each of the samples were extrapolated on the standard graph in order to get the concentrations in mg/100 ml. Out of the two organisms (which isolated in the beginning) fungus (Mucor) gave the best result.

**pH:**

At pH 7, very good results of degradation are shown by organisms 2 (Staphylococcus spp.)

At pH 3, moderate degradation are shown by organism 3 (Bacillus spp.)

At pH 5, maximum degradation are shown by organism 4 fungus (Mucor spp.)

At pH 9, good degradation are shown by organism 3 (Bacillus spp.)

**Temperature:**

Out of the four organisms Bacillus spp. gave the best degradation results. All the organisms showed the best degradation at 37°C and 45°C.

**Static and non-static condition**

The degradation is slightly more in shaking than in non-shaking tubes. The shaking culture tubes showed better degradation.

**Transformation**

Transformation was done using DH5α as host culture and transformed colonies with plasmids from the two different organisms were transferred to different plates and left for incubation for 24 hours. After incubation, growth of blue and white colonies was observed. The white colonies indicate the transformed colonies. The blue colonies indicate the transformed colonies. The blue colonies indicate the untransformed colonies. The transformed mixture was plated onto LB plates with pesticide concentration of 1% ziram. It was found that...
the competent cell of DH5α were successfully transformed as there was degradation of ziram.

DAVIDSON J. Genetic exchange between bacteria in the environment. Plasmid. 1999;42:73-91

High Performance Liquid Chromatography

The 30th day sample inoculated with organisms was analysed for HPLC test.


CONCLUSION

The present study suggests that the isolated organisms are able to utilize ziram as energy source since no other nutrients were available or were present in limited quantity. Hence, they degrade ziram.

CONFLICT OF INTREST

The authors have no conflict of interest
ACKNOWLEDGEMENT

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