International Scholars Journals

International Journal of Environmental Biology Research ISSN 9318-218X Vol. 5 (6), pp. 238-242, September, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Sumbul Fathima. M[1] and Priya Iyer[2]

[1]Department of Biotechnology, Women`s Christian College, Chennai- 60006, India.
[2]PG and Research Department of Biotechnology, Women's Christian College, Chennai, India.

Accepted 04 August, 2018

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 comycetes 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).

Corresponding author E-mail: brajuraj@yahoo.com

'Study of the degradation of pesticides on loaded seeds by ozonation', Journal of Environmental Chemical. APHA, Standard methods for the examination of water and wastewater, 20th edition (American Public Health Association/American water works association/water environment Federation, Washington, DC) 1998.

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).

Figure 1. Microorganisms isolates.

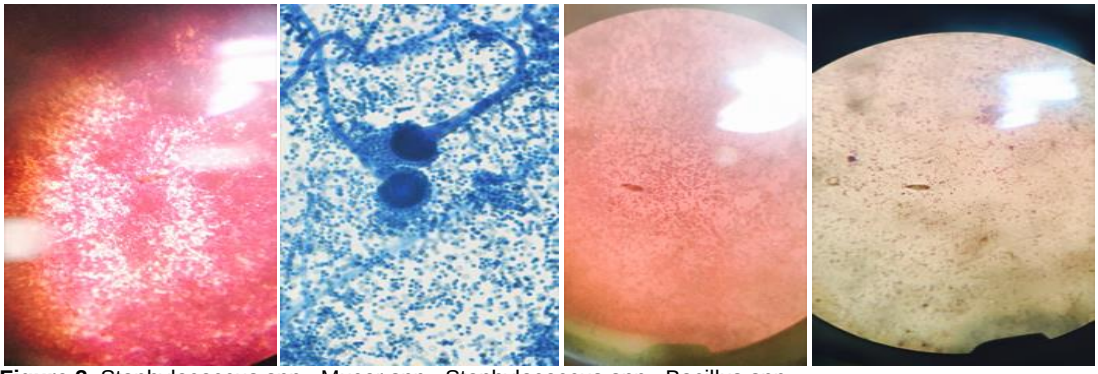


Figure 2. Staphylococcus spp., Mucor spp., Staphylococcus spp., Bacillus spp.,

Table 1. Biochemical tests

Biochemical tests	**Sample I**	**Sample 2**	**Sample 3**
Oxidase test	Negative	Negative	Positive
Catalase test	Positive	Positive	Positive
Citrate utilization test	Positive	Positive	Positive
Methyl red test	Negative	Negative	Positive
Vogesprosksuer test	Negative	Negative	Positive
Triple sugar ion test	Positive	Positive	Positive
Indole test	Negative	Negative	Negative
Coagulase test	Positive	Positive	Negative
Gelatine test	Negative	Negative	Positive
Identification	*Staphylococcus spp.*	*Staphylococcus spp.*	*Bacillus spp.*

Durai r., Ng P.C., Hoque H. Methicilin-resistant *staphylococcus aureus*: an updte. 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 (5^0C, 15^0C, 37^0C, 45^0C), 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

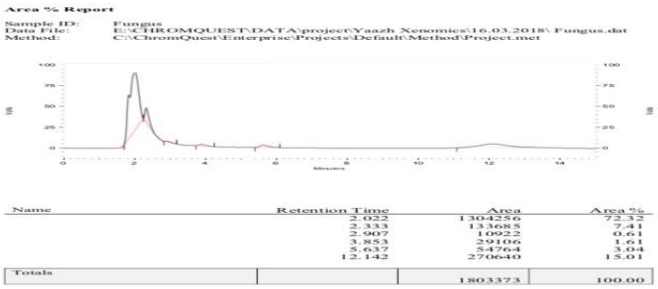


Figure 3. HPLC analysis of *Mucor*at pH5.

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.
http://www.volusol.com

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:

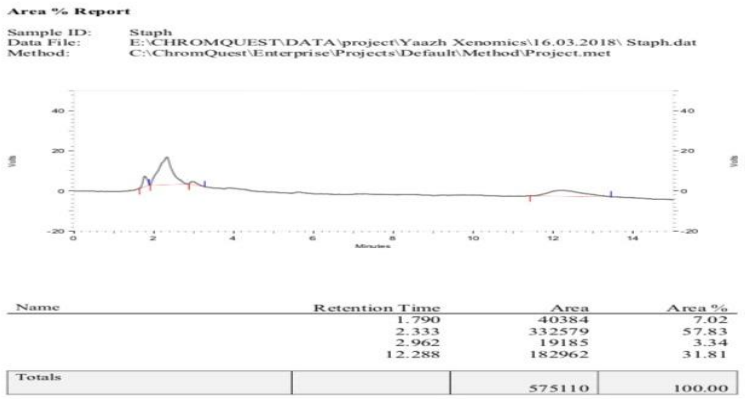
Out of the four organisms *Bacillus spp.* gave the best degradation results. All the organisms showed the best degradation at 37^0C and 45^0C.

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

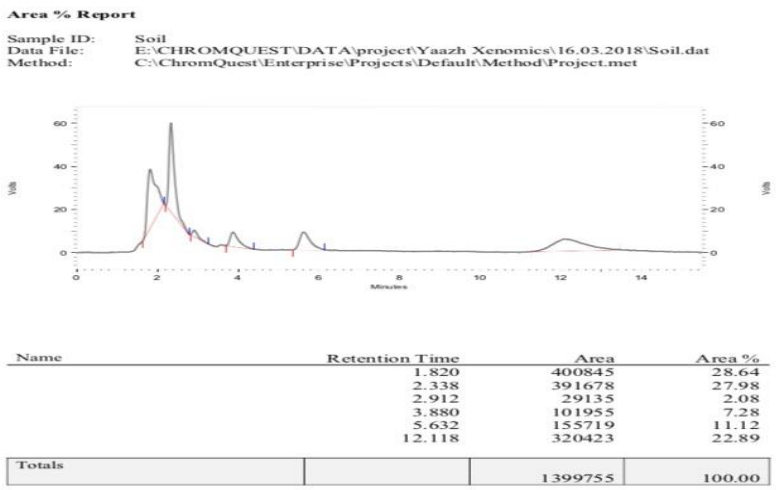
Transformation was done using DH5α as host culture and transformed colonies with plasmids from the two different organisms were transferred to different plates and left for incubation for 24 hours. After incubation, growth of blue and white colonies was observed.
The white colonies indicate the transformed 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



Area % Report

Sample ID: Staph
Data File: E:\CHROMQUEST\DATA\project\Yaazh Xenomics\16.03.2018\ Staph.dat
Method: C:\ChromQuest\Enterprise\Projects\Default\Method\Project.met

Name	Retention Time	Area	Area %
	1.790	40384	7.02
	2.333	332579	57.83
	2.962	19185	3.34
	12.288	182962	31.81
Totals		575110	100.00



Area % Report

Sample ID: Soil
Data File: E:\CHROMQUEST\DATA\project\Yaazh Xenomics\16.03.2018\Soil.dat
Method: C:\ChromQuest\Enterprise\Projects\Default\Method\Project.met

Name	Retention Time	Area	Area %
	1.820	400845	28.64
	2.338	391678	27.98
	2.912	29135	2.08
	3.880	101955	7.28
	5.632	155719	11.12
	12.118	320423	22.89
Totals		1399755	100.00

Figure 4. HPLC analysis of sample at non static condition with *Staphyloccus spp*. and sample at 45^{0}C with *Bacillus pp.*

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.

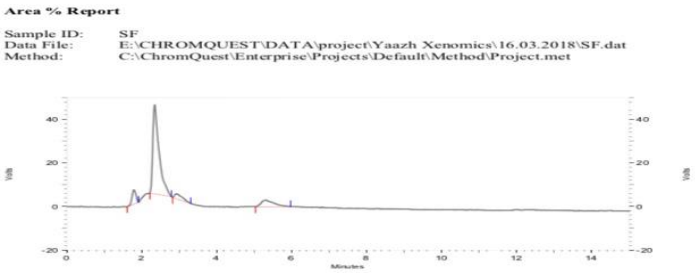
J.J. ZHANG, L.G. ZANG, J.E ZHANG, G.W. CUI and L.ZHOU., BULL. Chem, soc. Ethiop. 23 (2009) 97

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



Name	Retention Time	Area	Area %
	1.802	47042	7.40
	2.348	478637	75.25
	2.943	36407	5.72
	5.320	73979	11.63
Totals		636065	100.00

Figure 5. Day 1 sample of the ziram with *Staphylococcus spp.*

ACKNOWLEDGEMENT

The authors are thankful to Yaazh Xenomics support, Chennai, Tamil Nadu, India for their support in carrying out the work.

REFERENCES

Haque M.M, 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.

Food and Drug Administration (1986). The FDA Surveillance Index. Bureau of Foods, Department of Commerce, National Technical Information Service, Springfield, VA.

Marc Bourgin., Joe L Albet, Fre De Ricviolleau (2013). 'Study of the degradation of pesticides on loaded seeds by ozonation', Journal of Environmental Chemical Vol. 1, pp. 1004-1012.

APHA, Standard methods for the examination of water and wastewater, 20th edition (American Public Health Association/American water works association/water environment Federation, Wshington, DC) 1998.

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

Davidson J (1999). Genetic exchange between bacteria in the environment. Plasmid.42:73-91

J.J. Zhang, L.G. Zang, J.E Zhang, G.W. Cui, L. Zhou., Bull. Chem., soc. Ethiop. 23 (2009) 97

National Library of Medicine (1993). Hazardous Substances Databank. TOXNET, Medlars Management Section, Bethesda, MD.

National Research Council (1977). Drinking Water and Health, Advisory Center on Toxicology, Assembly of Life Sciences. Safe Drinking Water Committee, National Academy of Sciences, Washington, DC.

National Toxicology Program (1983). Carcinogenesis Bioassay of Ziram (CAS No. 137-30-4) in F344/N Rats and B6CF1 Mice (Feed Study). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Technical Report Series No. 238.