PROJECT REPORT (BSCC3051)

ON

Using the various bio chemical indicators , the efficiency of chlropyrifas ethyl remediation by methyloacterium radiotoleras and micro bacterium arthrosphaeral was assessed

Submitted in Partial Fulfilment of the Requirement for the degree of

B.Sc. (Hons) Chemistry

Submitted By

Harshi Aggarwal

(19SBAS1020004)

BSc .Chemistry (Hons.) (Semester VI)

Under the Supervision Of

Dr. SHAFAT AHMAD KHAN

Associated Professor, Division of Chemistry, Department of Basic Sciences

Galgotias University, U.P., India

&

Co-supervision of

MR. ASHWANI SHARMA

(MANAGER OF IRACHEM PVT LTD ,U.P, INDIA)

School of Basic and Applied Science

GALGOTIAS UNIVERSITY, U.P.

MAY 2022

GALGOTIAS UNIVERSITY

(Established under Galgotias University Uttar Pradesh Act No. 14 of 2011)

School of Basic and Applied Science

CERTIFICATE

This is certify that Ms. Harshi Agarwal has carried out her major project work entitled "**Using the various bio chemical indicators ,the efficiency of chlropyrifas ethyl remediation by methyloacterium radiotoleras and micro bacterium arthrosphaeral was assessed**" under my supervision . This work is fit for submission for the award of Bachelor's degree in chemistry .

Dr. Arvind kumar Jain
Dean
School of Basic & Applied Science
Galgotias University, Greater Noida

Dr. Shafat Ahmad Khan
Associated Professor
School of Basic & Applied Science
Galgotias University,
Greater Noida

IRACHEM LTD

C-5, UPSIDC Industrial Area,
Gajraula, Distt. Amroha,
U.P -244235
Ph.: 9412136718
Email: irachem.gajraula@gmail.com

CERTIFICATE

This is certify that Ms. Harshi Agarwal has carried out her major project work entitled "Using the responses of various bio chemical indicators ,the efficiency of chlropyrifas ethyl remediation by methyloacterium radiotoleras and micro bacterium arthrosphaeral was assessed" under my supervision from 16th January to 31st may -2022. This work is fit for submission for the award of Bachelor's degree in chemistry .

I wish her all the best for her upcoming career .

Regards

Mr. Ashwani Sharma

GALGOTIAS UNIVERSITY

(Established under Galgotias University Uttar Pradesh Act No. 14 of 2011)

School of Basic and Applied Science

Candidate declaration

I hereby declare that the dissertation entitled “Using the various bio chemical indicators , the efficiency of chlropyrifas ethyl remediation by methyloacterium radiotoleras and micro bacterium arthrosphaeral was assessed” submitted by me in partial fulfillment for the degree of B.Sc. (Hons) Chemistry to the Division of Chemistry, Department of Basic Sciences, School of Basic and Applied Science, Galgotias University, Greater Noida , Uttar Pradesh, India is my original work. It has not been submitted in part or full to this University of any other Universities for the award of diploma or degree.

TABLE OF CONTENT

Notations

1. **ROS:-** Reactive oxygen species
2. **CPF** :- Chlorpyrifos-ethyl
3. **SOD** :-Superoxide dismutase
4. **GSH-Px:-** Glutathione peroxidase
5. **CAT** :- Catalase
6. **G. pulex** :- Gammarus pulex
7. **BOD:-** Biochemical oxygen demand
8. **COD:-** Chemical oxygen demand
9. **PBS :-** Phosphate buffered saline
10. **PASW :-** Predictive Analytics Software

ACKNOWLEDGEMENT

This project has required a lot of effort from me. However, without the kind support and assistance of many individuals and organisations, it would not have been feasible. I would want to express my heartfelt gratitude to each and every one of them.

I owe a debt of gratitude to **Dr.Shafat Ahmad Khan** for their advice and ongoing supervision, as well as for supplying vital project information and assistance in completing the project.

It gives me immense pleasure to express my profound gratitude, gratefulness and indebtedness to reverend teacher and supervisor **Mr. Ashwani Sharma, Manager of Irachem pvt ltd, agriculture department ,GAJRAULA UP** for his untiring help, constant encouragement, worthy supervision, and providing me necessary facilities to carry out my work.

I submit my deep sense of gratitude to Dr. A. K. Jain, SBAS, Galgotias University and all other faculty members for encouraging me to work with determination and guiding me with my academics.

On a personal note, I have no words to express my abundant inexplicable affectionate gratitude to my parent for their catalytic role and all my loved ones whose patience and support has been an invaluable source of strength.

1. ABSTRACT

Abstract

This study examines the detoxifying and antioxidant enzyme responses of Gammarus pulex before and after biodegradation/bioremediation by Methylobacterium radiotolerans and Microbacterium arthrosphaerae after exposure to chlorpyrifos-ethyl pesticide.G. pulex treated to chlorpyrifos-ethyl had its cytochrome, glutathione S-transferase, catalase, and superoxide dismutase activities measured using commercial ELISA kits before and after bioremediation/biodegradation by these two bacteria for 24 and 96 hours.Before and after bioremediation/biodegradation, the activity of the catalase enzyme was reduced based on chlorpyrifos-ethyl, and then elevated again.After 4 days of exposure to chlorpyrifos-ethyl, superoxide dismutase activity increased ($p > 0.49$).

Superoxide dismutase enzyme activity decreased for 24 hours after bioremediation ($p > 0.49$) before rising for 4 days (p 0.49) Before and after the process, there were no statistical alterations in cytochrome P450 1A1 enzyme activity ($p > 0.4$).. During the activity of glutathione S-transferase in 24 hours, no significant variations were found ($p > 0.49$).After 96 hours of exposure to chlorpyrifos-ethyl, glutathione S-transferase activity increased.After bioremediation, glutathione S-transferase activity increased considerably higher (p 0.49).The findings showed that G. pulex activities in Common biomarkers for measuring the success of chlorpyrifos-ethyl bioremediation with these two different species of soil microorganisms are superoxide dismutase, catalase, and glutathione S-transferase.

2 . INTRODUCTION

Introduction

Today, the increase of pesticides causes environmental problems (Anwar 1997).Pesticides boost production, protect stored plants, and limit disease vectors, but they have negative health impacts when humans are exposed to them in the workplace and/or in the environment.Insecticides alter enzyme activity and antioxidative defence mechanisms, according to research.Organophosphorus insecticides can cause toxicity by increasing reactive oxygen species (ROS) levels (Altuntas et al. 2003) Chlorpyrifos-ethyl (CPF) is an organophosphate insecticide commonly used in agriculture, forestry, and horticulture to defend against pests (Eaton et al. 2008) .

CPF is a significant insecticide that regularly affects animals and humans since it is routinely employed in agricultural fields and residences. The metabolite of CPF, chlorpyrifos-oxon, reveals the major harmful action of CPF. Cytochrome P450 converts CPF to chlorpyrifos-oxon . In previous studies, CPF was shown to influence the activity of antioxidant enzymes such superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). (Gultekin).et al. 2001). The majority of bacteria studied were involved in impurity detoxification and remediation in the receiving environment. They are able to decompose pollutants that have been removed from farming areas (Desaint et al. 2000). Several pesticide degradation enzymes may be found in soil bacteria, and they are present on plasmids, which is a common location for biodegradation genes (Laemmli et al. 2000).Biodegradable catabolic plasmids can destroy persistent organic contaminants significantly. The large number of such plasmids were discovered in soil bacteria strains such as Flavobacterium, Actinobacteria, Pseudomonas, Klebsiella , and others. Arthrobacter and (Sayler et al. 1990). Traditional pesticide bioremediation methods, including such landfilling, incineration, recycling, and pyrolysis, are unsatisfactory, and so they can lead in pesticide production(Dua et al. 2002) . In this case, biological treatment methods are recommended over conventional treatments because microorganisms can breakdown the materials.Most of them do so without releasing hazardous byproducts. Organophosphate insecticides are reported to create oxidative rate and increasing free radical production and reducing antioxidant activity. (Birkhoj et al. 2004).The catalase enzyme reduces hydrogen peroxide, while superoxide dismutase converts superoxide radical to hydrogen peroxide. water and oxygen (Cemeli et al. 2009)

Objectives

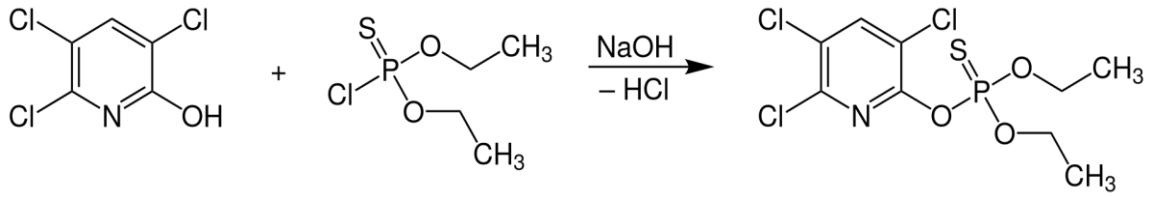
- First made the chlropyrifas ethyl 20%
- Determine the concentration of chlropyrifas ethyl.
- Degradation of chlropyrifas ethyl by various bio chemical indicators**.**
- Analyze the efficiency of chlropyrifas ethyl by using methyloacterium radiotoleras and micro bacterium arthrosphaeral

3. Methods and materials

Formulation of chlropyrifas ethyl

a. Technical Process

Chlorpyrifos(3) is manufactured industrially by reacting 3,5,6-trichloro-2-pyridinol (TCPy) (1) with O,O-diethyl phosphorochloridothioate. (2).



1 2 3

b. Mixing

Mixing of solvents (75%), emulsifiers(5%) and technical pesticides(20% or 50% acc to the concentration of the product) in tanks .

c .Testing

Test	Specification	Result contained
1. Appearance	The material shall be form of homogeneous &stable liquid.	Complete
2. Active ingredient	(19.00 to 21.00)%	20.00%
3. Emulsion free	Separation free	Passes
4. Acidity	0.050% max	0.036%
5. Cold test	Freeze free	Passes
6.Flash point	ABOUT 24*C	43*C

1. The identification of species

The bacteria were discovered in a cornfield in Turkey's Thrace/Marmara region To begin, 14-21 cm of agricultural soil samples weighing 9–11 g were gathered. and placed in sterile jars (zelles et. al 1991). A 0.1 ml (single drop) of the farm sample taken was reduced to 104 in isotonic soaking 0.8 percent salt (sodium chlorate) and seeded into a sterile plate. Measure the gel and store it at 28°C for five days for growing days, and the letters B1 and B2 were assigned. Hot Start Phire II It was identified using DNA polymerase. With bacterial 16S ribosomal primers, PCR bands of varied lengths (1000–3000 bp) were employed. "AGA GTT TGA TCC TGG CTC AG" was determined for 16S rRNA forward primers, and "ACG GCT ACC TTG TTA CGA CTT" was determined for 16S rRNA reverse primers. With an accuracy rate of above 90%, B1 and B2 species were determined as JCM2831 for M. radiotolerans and FN870023 for M. arthrosphaerae using BLAST software.

3. Insects and experimental procedure

Munzur River (39.156820 N, 39.499640 E) provided the model organisms (Fig. 1). Individuals of G. pulex were transported in plastic bottles . For 15 days previous to the experiment, the animals were kept in a 20-liter tank with a 12:12 light:dark cycle and given willow leaves. The organisms in a similar intermolt stage with around 10 mm in length were picked using a binocular (De Lange et al. 2006). With 3-trial replication, the tanks had ten people. Aquariums with a total capacity of 1 L were chosen. The organisms were not fed during the experiments. During the experiment, the dead subjects were removed. GST, CYP1A1, SOD, and CAT were classified into four groups.

1. Control group (distilled water) (A column)

2. B column: 1.25 ppb, 500 ml CPF (existing bacteria have no bioremediation activity).

3. C column: 1.25 ppb, 500 ml of CPF, and consortia of M. radiotolerans and M. arthrosphaerae (includes approximately 2 × 109 colony-forming unit ((CFU)/ml), initial bioremediation/biodegradation stage)

4. D column: 1.25 ppb, 500 ml of CPF, and consortia of M. radiotolerans and M. arthrosphaerae (2 ml), 8th day of bioremediation, last stage The G. pulex individuals (n: 10 in each group) were uncovered to these groups for 24 and 96 h.

Fig. 1 Collecting of *G. Pulex*

Fig 1. Collecting of g. pulex (De Lange et al. 2006),

4. Biochemical analysis :-

Before the biochemical assays, tissues were firstly homogenized and weighed after 1/5 PBS was added. Once the homogenization step was finished, the samples were centrifuged at 17000 rpm for 15 min. The obtained supernatants were stored at − 80 °C deep freeze until the analyses were done.

5. Statistical analysis :-

PASW Statistics 18.0 used for analyzing data. To assess the statistical differences from groups, the Duncan's multiple range tests and one-way ANOVA were used under the same exposure time ($abcp < 0.05$). Two-tailed independent T test was selected to reveal out the differences between the exposure times (24 and 96 h) in the same group ($*p < 0.05$)

4. RESULTS

Results

Reduction of COD and BOD5

The two bacteria were able to complete their bioremediation/biodegradation steps in media with CPF since the insecticide was in the early stages of the cod value in 100ml mixed media . To 1.25 ppb 100 ml CPF solutions, 2 ml increased bacterial species were introduced. The COD and BOD5 reductions of the media are given in Fig. 2.Consortia of m radiotolerans and m.arthosphera achieved reduction rates of over 94 percent after 8 days based on the results of cod and bod5 assay

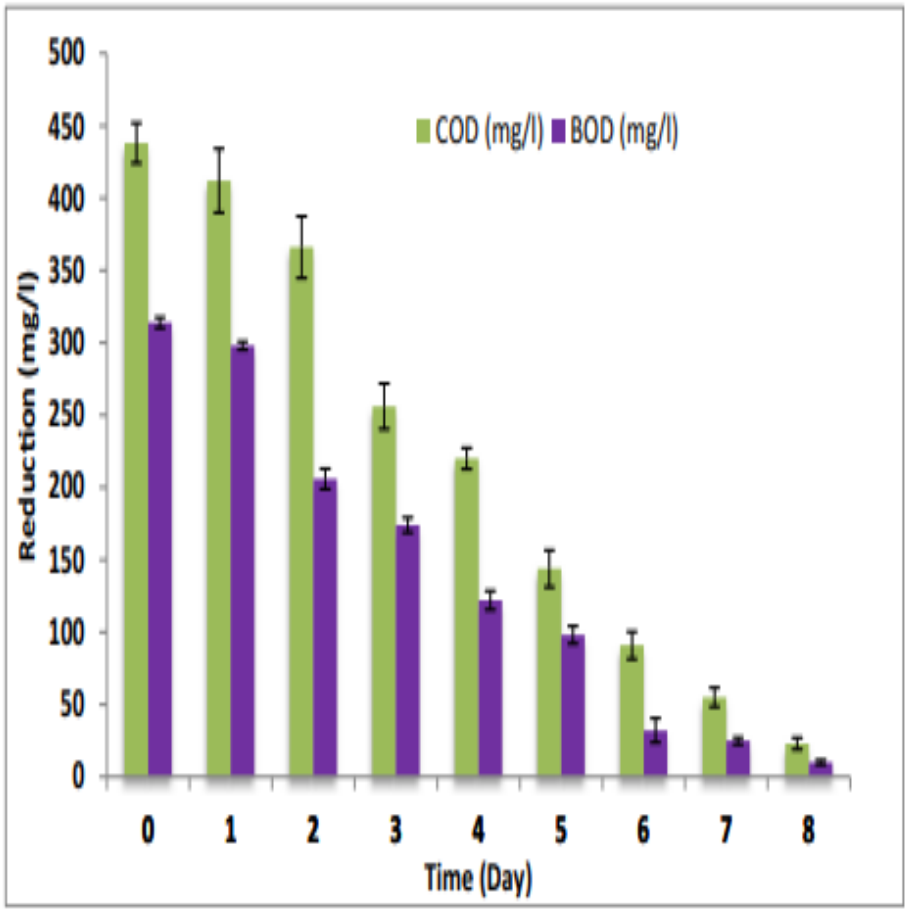


Fig. 2 Reduction of COD and BOD_5

5. Conclusion

Conclusion

Herein, we make chlropyrifas ethyl insecticide in industry and then testing in their laboratory . cps is used in crops to kill insects and worms which is harmful for crops. But Cps is make in two different concentration 20% and 50%**..**M. radiotolerans and M. arthrosphaerae were able to efficiently remediate CPF, indicating that this bioremediation improved G. pulex's oxidative stress. G. pulex SOD, CAT, and GST activities are effective biomarkers for determining the efficiency of CPF remediation with Methylobacterium radiotolerans and Microbacterium arthrosphaerae. Our following biotechnological technique is quite effective in decontaminating aquatics that have been contaminated with organic pollutants, based on the findings of the studies.Both M. radiotolerans and M. arthrosphaerae have a comparable pesticide biodegradation pathway and are genetically quick to respond to chemical contaminants. Microorganisms' breakdown and detoxifying capacity is being exploited to remove pesticide pollution from water. In G. pulex, it was discovered that CPF increases oxidative stress, and biochemical indicators were changed by exposure time at various degrees.

6 References

1. Agrawa A, Sharma B (2010) Pesticide induced oxidative stress in mammalian systems. Int J Biol Med Res 1(3):90–104

2. Aksu O, Yildirim NC, Yildirim N, Danabas D, Danabas S (2015) Biochemical response of crayfish Astacus leptodactylus exposed to textile wastewater treated by indigenous white rot fungus Coriolus versicolor. Environ Sci Pollut Res 22:2987–2993

3. Altuntas I, Delibas N, Doguc DK, Ozmen S, Gultekin F (2003) Role of reactive oxygen species in organophosphate insecticide phosalone toxicity in erythrocytes in vitro. Toxicol in Vitro 17(2):153–157

4. Anwar WA (1997) Biomarker and human exposure to pesticides. Environ Health Perspect 105:801–806

5. APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington DC

6. Arias-Estevez M, Lopez-Periago E, Martinez-Carballo E, Simal-Gandara J, Mejuto JC, Garcia-Rio L (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric Ecosyst Environ 123:247–260

7. Bacchetta C, Rossi AS, Ale A, Campana M, Parma J, Cazenave J (2014) Combined toxicological effects of pesticides: a fish multibiomarker approach. Ecol Indic 6:532–53

8 Sparks, Thomas C.; Crossthwaite, Andrew J.; Nauen, Ralf; Banba, Shinichi; Cordova, Daniel; Earley, Fergus; Ebbinghaus-Kintscher, Ulrich; Fujioka, Shinsuke; Hirao, Ayako; Karmon, Danny; Kennedy, Robert; Nakao, Toshifumi; Popham, Holly J.R.; Salgado, Vincent; Watson, Gerald B.; Wedel, Barbara J.; Wessels, Frank J. (2020). "Insecticides, biologics and nematicides: Updates to IRAC's mode of action classification - a tool for resistance management". Pesticide Biochemistry and Physiology. Elsevier

9 O. Gaonkar, I. M. Nambi and G. Suresh Kumar, Biodegradation kinetics of dichlorvos and chlorpyrifos by enriched bacterial cultures from an agricultural soil, Bioremediation Journal, 2019, 23, 259–276..

10 A. K. Maurya, A. Kumar, K. Sharma and P. E. Joseph, Organochlorine pesticides concentration in the ground water from regions of extensive agriculture in LakhimpurKheeri, Uttar Pradesh India, Global Journal of Engineering, Design and Technology, 2013, 2, 24–30.

11 J. M. Hatcher, K. D. Pennell and G. W. Miller, Parkinson's disease and pesticides: a toxicological perspective, Trends in Pharmacological Sciences, 2008, 29, 322–329

12 Thomas A. Unger (1996). Pesticide Synthesis Handbook. p. 313. ISBN 0-8155-1853-6.

13 "Dursban Pro specimen label" (PDF). Archived from the original (PDF) on 24 August 2009.

14 ."Strike-Out 500WP label" (PDF). Archived from the original (PDF) on 11 March 2011

15 . In: Cobbs AH, Kirkwood RC (eds) Herbicides and Their Mechanisms of Action. Sheffield Academic, Sheffield, pp 25–37

16 Birkhoj M, Nellemann C, Jarfelt K, Jacobsen H, Andersen HR, Dalggard M, Vinggaard AM (2004) The combined antiandrogenic effects of five commonly used pesticides. Toxicol Appl Pharmacol 15(201): 10–20

17 . Boryslawskyj M, Garrood AC, Pearson JT (1988) Elevation of GST as a stress response to organochlorine compounds in Sphaerium corneum. Mar Environ Res 24:101–104

18 . Boschin GD, Agostina A, Arnoldi A, Marotta E, Zanardini E, Negri M, Valle A, Sorlini C (2003) Biodegradation of chlorsulfuron and metsulfuron- methyl by aspergillus niger in laboratory conditions. J Environ Sci Health B 38(6):737–746

19 Cemeli E, Baumgartner A, Anderson D (2009) Antioxidants and the Comet assay. Mutat Res Rev Mutat Res 681(1):51–6

20 Cremlyn R (1991) Agrochemicals: preparation and mode of action. Wiley, Chichester

21 De Lange HJ, Noordoven W, Murk AJ, Lürling MFLLW, Peeters ETHM (2006) Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat Toxicol 78:209–216

22 Demirci O, Güven K, Asma D, Öğüt S, Uğurlu P (2018) Effects of endosulfan, thiamethoxam, and indoxacarb in combination with atrazine on multi-biomarkers in Gammarus kischineffensis. Ecotoxicol Environ Saf 147(218):749–758

23 Desaint S, Hartmann A, Parekh NR, Fournıer JC (2000) Genetic diversity of carbofuran-degrading soil bacteria. FEMS Microbiol Ecol 34(2): 173–180

harshi report2

ORIGINALITY REPORT

7%
SIMILARITY INDEX

PRIMARY SOURCES

1	Gokhan Onder Erguven, Şule Tatar, Osman Serdar, Nuran Cikcikoglu Yildirim. "Evaluation of the efficiency of chlorpyrifos-ethyl remediation by Methylobacterium radiotolerans and Microbacterium arthrosphaerae using response of some biochemical biomarkers", Environmental Science and Pollution Research, 2020 Crossref	75 words — 6%
2	en.wikipedia.org Internet	19 words — 2%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 14 WORDS