



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 Issue: IV Month of publication: April 2017

DOI:

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

Isolation and Screening of Organophosphorous Pesticides (Malathion) Degrading Organisms from Soil

Hiren Prajapati¹, Mayur Gahlout², Poonam Chauhan³, Akanksha Singh⁴, Kiran Yadav⁵

^{1,2,3,4,5}Department of Microbiology, KBS Commerce and NATARAJ Professional Sciences College, VAPI. Dist. Valsad 396195, Gujarat, India

Abstract: Organophosphate like Malathion is used for control of household and agricultural pests. High levels of malathion contaminates soil, water and aquatic ecosystems. The wide spread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment. Present study reports the isolation, morphological and Estimation of Malathion degrading bacteria. Soil samples collected from various agriculture field, Garage field, Garden, etc (Vapi and Valsad Region). Malathion was used as a sole source of carbon to enumerate Malathion degrader. Total 15 isolates (AK1...AK15) were obtained from the soil sample, using Mineral Salt Medium. AK5 isolate as shows maximum percentage degradation at 100 ppm concentration of Malathion. The analysis shows that isolated organism belongs to Bacillus species.

Keywords: Malathion, Organophosphate(OP), Biodegradation

I. INTRODUCTION

Pesticides are organic compounds manufactured and used control destructive pests such as insects, plant disease organism , unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage , transparent of marketing of food, agriculture commodities, wood and wood products or animal food stuff, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (S.R.Shinde et al., 2015).

Pesticides are indispensable to modern agriculture. Some of the main agricultural products are parathion, methyl parathion, chlorpyrifos, malathion, monocrotophos, quinalphos and dimethoate. Microorganisms are used in bioremediation of environmental pollutants. Bacteria and fungi are capable of degrading malathion. Malathion can be degraded or detoxified using physical, chemical, or biological methods (A.RatnaKumari et al., 2012). Few bacterial species like Bacillus spp., Pseudomonas spp., Staphylococcus spp., Enterobacter spp., Klebsellasp noted to degrade malathion in recent studies.(Karishma Baishya et al., 2015)

Biodegradation is common method for the removal (degradation and detoxification) of organophosphate pesticides because of its low cost and low collateral destruction of indigenous animal plant organisms (Liu et al., 2007). Bacterial degradation is considered to be a major factor determining the fate of Malathion and other organophosphate pesticides in the environment. Degradation of pesticides is usually a combination of a number of processes, including microbial degradation and chemical hydrolysis and is also influenced by some physicochemical properties such as temperature, pH and carbon and nitrogen source(Soni Yadav et al., 2015).

II. MATERIALS AND METHOD

A. Screening, Isolation and Purification of Malathion Degrading Bacteria

For isolation of malathion degrading bacteria, one gram of each soil sample were suspended into 9 ml of sterile distilled water and serially diluted up to 6 folds. From the last three dilution tubes take 0.1 ml and spread into the mineral salt agar medium containing malathion as sole source of Carbon and Nitrogen. Plates were incubated at 37⁰C for 48 hours. The isolated colonies were grown on BHM agar plate.

International Journal for Research in Applied Science & Engineering Technology (IJRASET)

B. Storage and Maintenance of Pure Culture

The isolated organisms were streaked on MSM agar slants. The slants were incubated at 30°C for 48 hrs. The pure culture is then stored in refrigerator at 4°C and sub cultured periodically

C. Degradation Experiment

1) *Inoculum Preparation*: Inoculum was prepared by transferring preserved culture and growing the cells in 100 ml Erlenmeyer flask containing 50 ml nutrient broth. The flasks were incubated at 30°C for 24 hrs. The freshly grown 24 hrs old culture with 1.0 O.D. at 600 nm is used as Inoculum to inoculate degradation medium MSM broth containing 100 ppm malathion.

D. Media for Degradation

Composition of medium for degradation [K_2HPO_4 6.0gms, KH_2PO_4 1.0gm, NH_2NO_3 1.0gm, $MgSO_4 \cdot 7H_2O$ 0.1gm, NaCl 5.0 in 1 liter of distilled water ,pH of medium was adjust to 7.0 using pH meter, and medium was autoclaved] containing 100ppm malathion

E. Inoculation of Medium for Degradation

The sterilized medium was inoculated with 100 ppm malathion and 1% (v/v) of 24 hrs old culture. The inoculated flask was allowed to incubate at 30°C for 48 hrs. The sample was withdrawn at 24 hrs of interval and supernatant was subjected to centrifugation at 5,000 rpm for 20 min and degradation rate was determined

F. Estimation of Malathion

1ml of sample, 1 ml of alcoholic potassium hydroxide and 10 ml of 0.1N potassium bromate was added. Then 0.5ml of 1:1 nitric acid and 2ml of double distilled water are added to give orange yellow color. The solution was kept aside for 5 min before taking absorbance and absorbance was measured at 415nm against reagent blank. (*Eurasian J Anal Chem* 8(3):131-135,2013). Degradation was quantitatively analyzed by measuring the absorbance of the supernatant at maximum absorption wavelength, λ_{max} of respective pesticides. Degradation was calculated by using the equation:

$$\% \text{ Degradation} = (A - B) / A \times 100$$

Where, A is initial absorbance of control malathion (initial absorbance) and B is observed absorbance of degraded malathion (final absorbance).

G. Screening of Isolates having Degradation Activity

Bacteria were screened on the basis of degradation capability using malathion. Bacteria which shows higher degradation activity is selected and further study is carried out using it. The screening of isolates degrading malathion was measured as decrease in optical density using spectrophotometer. Degradation of malathion was done by promising isolate and effect of incubation period was optimized

H. Effect of Incubation Temperature

In present study, the effect of incubation period was determined by incubating medium at different incubation temperature such as 15-45° C. In Erlenmeyer flask, 100 ml of MSM broth, 100 ppm malathion and 1% inoculum were added and incubated for 48 hr. The sample was withdrawn from medium at respective time interval and subjected to centrifugation at 5,000 rpm for 20 min and the supernatant was used for determination of degradation.

III. RESULT AND DISCUSSION

Total 25 isolates were screened for its capability of degrading Malathion. 15 isolates capable of degrading Malathion were isolated from soil samples with a history of pesticide application. All these isolates were labeled as AK-1 to AK-15. All 15 isolates were further purified and stored after streaking on MSM agar plate. Bacterial isolate AK5 showed maximum degradation (76.54%) of Malathion within 48 hrs of incubation (Fig. 3.1) at 30°C under static condition. Followed by AK5, bacterial isolate AK2 & AK8 shows 70.0 % & 69.98% phenol degradation, respectively. Thus, isolate AK5 was selected for further study as it exhibit highest degradation of Malathion.

International Journal for Research in Applied Science & Engineering Technology (IJRASET)

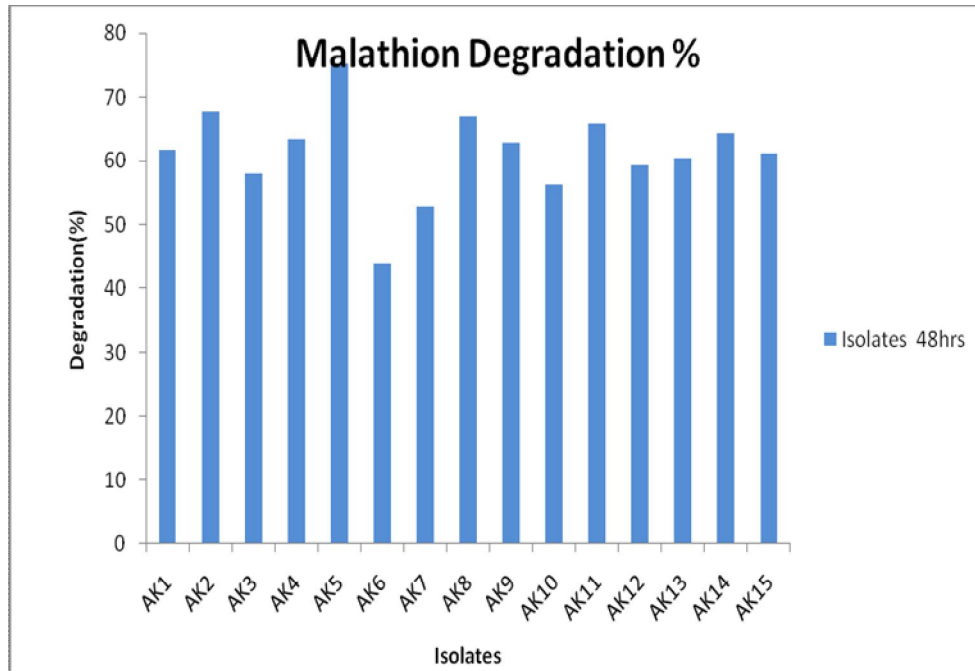


Figure-1 Malathion Degradation

Temperature is one of the important factors affecting the growth and activity of microorganisms. Effect of varying temperature on Malathion degradation was studied by incubating the inoculated experimental flask in the temperature range of 15- 45° C. The sample was with drawn after 48 hrs of incubation, centrifuged at 5000 rpm for 20 min and supernatant was used to determine Malathion degradation. The optimum temperature for maximum degradation of Malathion was recorded at 30° similar result have been reported by C.Sony Yadav et al., (2015) and KayLynn Newhart et al.,(2006).

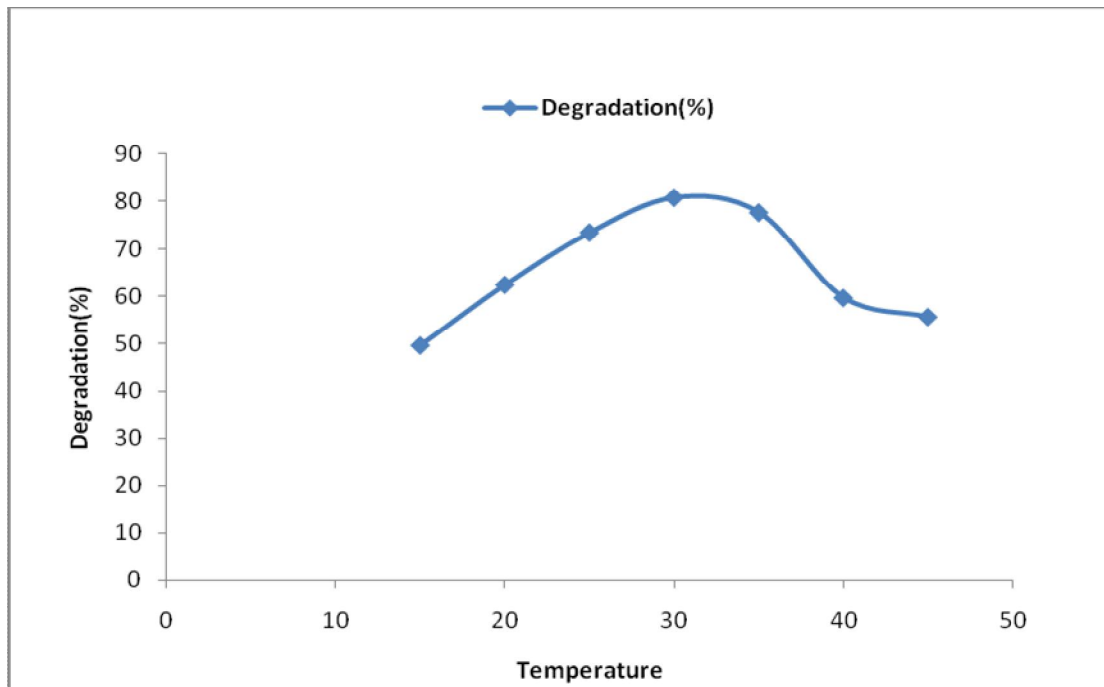


Fig Effect of Temperature on Malathion degradation

International Journal for Research in Applied Science & Engineering Technology (IJRASET)

IV. CONCLUSION

The study shows that the isolated bacterium AK5 is able to degrade the Malathion, so that this organism is used as a biological agent for the *in situ* bioremediation of Malathion contaminated soil. The present study is done only on the morphological and cultural characterisation of isolated bacteria. These eco-friendly bacteria can be used and motivate the farmers to use natural biological pesticides.

V. ACKNOWLEDGEMENT

We would like to thank our principal and all the faculty members of K.B.S. Commerce & Nataraj Professional Sciences College for providing all the laboratory facilities and for their support provided at all the steps of this study.

REFERENCES

- [1] N. V. S. VENUGOPAL*, B. SUMALATHA AND SYEDABANO, Spectrophotometric Determination of Malathion in Environmental Samples, Department of Chemistry, G.I.T, GITAM University Rushikonda, Vsakhapatnam-530045.A.P,India, ISSN: 0973-4945; CODEN ECJHAO E-Journal of Chemistry, <http://www.ejchem.net> 2012, **9(2)**, 857-862
- [2] Karishma Baishya and Hari Prasad Sarma, Advances in biodegradation of organophosphorus pesticides, Department of Environmental Science, Gauhati University, Guwahati, Assam, India. Scholars Research Library, Archives of Applied Science Research, 2015, **7 (4)**:37-43. (<http://scholarsresearchlibrary.com/archive.html>)
- [3] Metin DIÜRAK, FerdaÜ KAZANICI Kahramanmaraş S.t, Effect of Some Organophosphorus Insecticides on Soil Microorganisms. Ümam University, Faculty of Arts & Science, Department of Microbiology,46045, Kahramanmaraş – TURKEY, Turk J Biol 25 (2001) 51-58 © T.BÜTAK.
- [4] K. Visweswriah & M. Jayaram (1974) Colorimetric Method for the Estimation of Malathion, Agricultural and Biological Chemistry, 38:10, 2031-2033, DOI:10.1080/00021369.1974.10861453 To link to this article: <http://dx.doi.org/10.1080/00021369.1974.10861453> Published online: 09 Sep 2014.
- [5] A.Ratna Kumari1*, G. Jeevan1, M. Ashok1, Ch.Koteswara Rao2K. S. K. Vamsi1, Malathion degradation by Bacillus spp. isolated from soil 1. Department of Biotechnology, Bapatla Engineering College, Bapatla-522 101, A.P., India 2. Centre for Biotechnology, Department of Chemical Engineering, Andhra University, Vizag-530 003, A.P., India. IOSR Journal of Pharmacy ISSN: 2250-3013, www.iosrphr.org || Volume 2 Issue 4 || July-August 2012 || PP.37-42 37.
- [6] B.J. Bhadbhade, S.S. Sarnaik and P.P. Kanekar, Biomineralization of an organophosphorus pesticide, Monocrotophos, by soil bacteria, Microbial Sciences Division, Agharkar Research Institute, Pune, Maharashtra, India 363/11/01: received 16 November 2001, revised 19 March 2002 and accepted 3 April 2002.
- [7] Soni Yadav, Sitansu Kumar Verma and Hotam Singh Chaudhary, Isolation and Characterization of Organophosphate Pesticides Degrading Bacteria from Contaminated Agricultural Soil, Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, M.P.-474005, India. © This open access article is distributed under a Creative Commons Attribution (CC-BY) 3.0 license.
- [8] S.R.Shinde1*, M.V.Bhailume1, N.B.Patil1, N.N. Patil1 and V.S.Hamde2 Screening, Characterization and Identification of Soil Isolates for degradation of Organophosphorus group of pesticides (Dimethoate and Parathion). Int.J.Curr.Microbiol.App.Sci (2015) Special Issue-2: 240-244.
- [9] Sayed K. Goda • Iman E. Elsayed • Taha A. Khodair • Walaa El-Sayed • Mervat E. Mohamed. Screening for and isolation and identification of malathion-degrading bacteria: cloning and sequencing a gene that potentially encodes the malathion-degrading enzyme, carboxylestrase in soil bacteria, Received: 22 September 2009 / Accepted: 12 March 2010_ Springer Science+Business Media B.V. 2010
- [10] Maryam W. Aziz, American-Eurasian J. Agric. & Environ. Biodegradation of Malathion by Pseudomonas Spp. and Bacillus Spp. Isolated From polluted Sites in Egypt Sci., 14 (9): 855-862, 2014 ISSN 1818-6769, © IDOSI Publications, 2014 DOI: 10.5829/idosi.aejaes.2014.14.09.867.
- [11] Pesticide residues in food 2016, Joint FAO/WHO Meeting on pesticides residues, Report of the special session of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues Geneva, Switzerland, 9–13 May 2016.
- [12] Fang Zhu 1,2,* , Laura Lavine 2, Sally O'Neal 1, Mark Lavine 2, Carrie Foss 3 and Douglas Walsh 1 Insecticide Resistance and Management Strategies in Urban Ecosystems, Received: 26 October 2015; Accepted: 28 December 2015; Published: 6 January 2016
- [13] TOXICOLOGICAL PROFILE FOR MALATHION, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Public Health Service, Agency for Toxic Substances and Disease Registry, September 2003.
- [14] Tamer M.A. Thabit1,2, Medhat A.H. EL-Naggar2, Malathion degradation by soil isolated bacteria and detection of degradation products by GC-MS.1- Central Agric. Pesticides Lab. (CAPL), Agric. Research Center, Giza, Egypt 2- Research Central Lab., Grain Silos and Flour Mills Org. (GSFMO), Riyadh, KSA dr.tamerthabit@live.com ,doi:10.6088/ijes.2013030500017, INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES, Volume 3, No 5, 2013.
- [15] Wen Li, Ming Sun and Minzan Li, A Survey of Determination for Organophosphorus Pesticide Residue in Agricultural Products Key Laboratory of Modern Precision Agricultural System Integration Research of Education, China Agricultural University, China No. 17 Tsinghua East Road Haidian District, Beijing, 100083, P.R. China. Advance Journal of Food Science and Technology 5(4): 381-386, 2013 ISSN: 2042-4868; e-ISSN: 2042-4876 © Maxwell Scientific Organization, 2013.
- [16] A Ratna Kumari1*, K. Sobha2, K. Mounika1, G. J. Nageswara Rao1 and M. Ashok1, MOLECULAR CHARACTERIZATION OF BACTERIA CAPABLE OF ORGANOPHOSPHATE DEGRADATION, , Int. J. LifeSc. Bt & Pharm. Res. 2012.
- [17] Shuyan Deng a, Yao Chen a, Daosheng Wang b, Taozhong Shia, Xiangwei Wua, Xin Maa, Xiangqiong Li a, Rimao Huaa,*, Xinyun Tang b, Qing X. Li c, Rapid biodegradation of organophosphorus pesticides by Stenotrophomonas sp. G1, a Key Laboratory of Agri-food Safety of Anhui Province, Lab of Quality & Safety and Risk Assessment for Agro-products on Storage and Preservation (Hefei), Ministry of Agriculture, School of Resource and Environment, Anhui Agricultural University, Hefei 230036, China b School of Life Science, Anhui Agricultural University, Hefei 230036, China c Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 957822, USA. Journal of Hazardous Materials 297 (2015) 17–24.

International Journal for Research in Applied Science & Engineering Technology (IJRASET)

- [18] Tova A. Samuels and Sherine O. Obare, Advances in Analytical Methods for Organophosphorus Pesticide Detection, Department of Chemistry, Western Michigan University, Kalamazoo, Michigan United States of America, 2013.
- [19] INSECTICIDE FACT SHEET, JOURNAL OF PESTICIDE REFORM/ WINTER 2003 • VOL. 23, NO. 4.
- [20] ORGANOPHOSPHATES PROFILE GUIDE, ©2012 Metamatrix, Inc. All rights reserved 0212 42040
- [21] Ghosh Poorva G., Sawant Neha A., Patil S. N., and Aglave B. A., (2010), Microbial biodegradation of organophosphate pesticides, International journal of biotechnology and biochemistry, 6, pp 871-876.
- [22] Kamal Zienat M., Fetyan Nashwa A. H., Ibrahim M. A., and El-Nagdy S., (2008), Biodegradation and detoxification of Malathion by of Bacillus Thuringiensis MOS-5, Australian journal basic and applied sciences, 2, pp 724-732.
- [23] Kanekar Pradnya p., Bhadbhade Bharati J., Deshpande Neelima M., and Sarneck Seema, S., (2004), Biodegradation of organophosphorous pesticides, Proceedings of Indian National Science Academy, B70, pp 57-70.
- [24] Zeinat Kamal M., Nashwa A.H. Fetyan A., Mohamed A. Ibrahim, Sherif El-Nagdy, Biodegradation and Detoxification of Malathion by of Bacillus Thuringiensis MOS-5 Botany Department, Faculty of Science, Cairo University, Egypt. 1 Soil, Water and Environment Research in Statute, Agriculture Research Center, Egypt. 2 Agricultural Genetic Engineering Research Institute Agricultural Research Center Egypt. Australian Journal of Basic and Applied Sciences, 2(3): 724-732, 2008 ISSN 1991-8178.
- [25] K. Srilatha Srinivas¹, Janhavi Damani², Effect of Malathion on the Concentration of Primary Metabolites from Vigna radiata and Trigonella foenum-graecum, Bull. Env. Pharmacol. Life Sci., Vol 5 [7] June 2016: 34-38 ©2016 Academy for Environment and Life Sciences, India.



10.22214/IJRASET



45.98



IMPACT FACTOR:
7.129



IMPACT FACTOR:
7.429



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089  (24*7 Support on Whatsapp)