ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 8, pp.123-126, December, 2010

RESEARCH ARTICLE

BIODEGRADATION OF METHYL PARATHION AND MONOCHROTOPHOS BY *Pseudomonas aeruginosa* AND *Trichoderma viridae*

Balamurugan[1], K., Ramakrishnan[2], M., Senthilkumar[1], R. and Ignacimuthu[2*], S.

[1] Department of Biotechnology, Thanthai Hans Roever College, Perambalur, Tamilnadu, India- 621 212.
[2] Division of Plant Biotechnology, Entomology Research Institute, Loyola College, Chennai, India- 600 034.

Received 18th August, 2010; Received in revised form; 25th August, 2010; Accepted 27th September, 2010; Published online 1st Decemb, 2010

In the present study, *Pseudomonas aeruginosa* and *Trichoderma viridae* were evaluated for their potential to degrade pesticides such as monochrotophos and methyl parathion. Soil alone, soil with pesticides, and soil with microbes and pesticides were taken as experimental modules. After 30 days of treatment different morphometric and biochemical parameters were analyzed. Percent germination, shoot and root length, content of chlorophyll-a, and b, total chlorophyll, protein and carotenoids were highly reduced in pesticide alone treated soil. However when the pesticide treated soil was mixed with *Pseudomonas aeruginosa* and *Trichoderma viridae,* the above mentioned parameters were restored to near normal indicating their capacity to degrade the pesticides. The present investigation showed that *Pseudomonas aeruginosa* was more efficient in degrading monochrotophos and methyl parathion.

Key words: MCP, MP, *Pseudomonas aeruginosa, Trichoderma viridae, Vigna mungo.*

INTRODUCTION

The economy of India is largely dependent on the quality and quantity of its agricultural production. Better harvests require intensive cultivation, irrigation, fertilizers, and the use of chemicals to protect plants from pests and plant diseases. In India, 15–20% of all produce is destroyed by pests (Bhalerao and Puranik, 2007). This emphasizes the paramount importance of pesticides in India in preventing agricultural loss and enhancing production. The enormous use of pesticides, however, has added to environmental pollution (Hewitt, 1998).

Methyl parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate,MP) is an organophosphate insecticide and acaricide used to control boll weevils and many biting or sucking insect pests of agricultural crops (Adhya *et al*. 1981). It is synthesized from diethyl dithiophosphoric acid. After being ingested by insects, the parathion becomes oxidized by oxidases to give paraoxon, replacing the double bonded sulfur with oxygen. It kills insects by contact, stomach and respiratory action. It is highly toxic to non-target organisms including humans. Parathion is marketing worldwide by different companies and under different brand names. Monocrotophos [dimethyl-(E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate] is an organophosphorus, nonspecific systemic insecticide and acaricide, used to control common mites, ticks, and spiders by contact and stomach action. Its water-soluble nature helps to penetrate quickly into plant tissue (Tomlin, 1994). MCP is still widely used in India for the protection of cash crops such as cotton, sugarcane, groundnut, tobacco, maize, rice, soybeans, and vegetables (Vig *et al.,* 2001; Bhadbhade *et al.,* 2002).

Organophosphate insecticide with high oral and moderate dermal toxicity and a half-life of 14–21 days. The toxicologically relevant mode of action is the inhibition of choline esterase activities (Skripsky and Loosli, 1994). It is mobile in soil and hence has a potential for groundwater contamination. It is weakly absorbed by soil particles because of its hydrophilic nature, increasing a threat of groundwater contamination due to leaching (Singh and Singh, 2003). It is one of the most toxic substances ever developed and has been found to be highly toxic to birds with the LD50 being

***Corresponding author:** *entolc@hotmail.com*

0.9–6.7 mg kg-1 (IPCS, 1993). It is highly irritating to the eyes and produces incoordination, slurred speech, loss of reflexes, weakness, involuntary muscle contractions, and paralysis of the body (Anonymous, 1997). To date, bacterial transformations have been the main focus in research on organophosphate pesticide degradation. The objectives of the present study was to assess the effect of microbes on the degradation of MCP and MP by studying on seed germination, root and shoot length , chlorophyll, protein and carotenoids content in *Vigna mungo* L. Hepper.

MATERIALS AND METHODS

Chemicals

Technical-grade monocrotophos and methylparathion (Syngenta India Ltd., Mumbai, India) were used in this study. A stock solution of monocrotophos and methylparathion at a concentration of 10,000 mg^{-1} was prepared in distilled water. Working solutions were prepared from stock solutions. All other chemicals used were of analytical grade.

Microorganisms

Bacterial and fungal strains were obtained from the Microbial Type Culture Collection (MTCC) at Chandigarh. The pure cultures of *Pseudomonas aeruginosa* and *Trichoderma viridae* were grown on Nutrient Agar and Potato Dextrose Agar medium respectively. Then pesticide degrading ability of *Pseudomonas aeruginosa* (at 37°C for 24 to 48 h) and *Trichoderma viridae* (at 25°C to 30°C for 48 to 72 h) was screened based on the microbial growth on pesticide containing medium with various concentration such as 0.2ml, 0.4ml, 0.6ml and 0.8ml. Then the effect of pesticide on plant growth was analyzed using pot culture experiments. Soil alone, soil with pesticides and soil containing microbes along with pesticides were taken as experimental modules. After 30 days morphometric characters such as germination ability, shoot length and root length and biochemical parameters such as chlorophyll (Arnon, 1949) total protein (Lowery *et al.,* 1951) and carotenoids (Kirk and Allen,1965)were estimated.

RESULT AND DISCUSSION

The ability of *Pseudomonas aeruginosa* and *Trichoderma viridae* to degrade pesticides (Methyl parathion and Monochrotophos) was analyzed. *Pseudomonas aeruginosa* promoted higher growth in methyl parathion contained soil. Moderate growth rate was noted in monochrotophos contained soil.

Table 1. Degradation of monochrotophos by *Pseudomonas aeruginosa* and *Trichoderma viridae* and their effect on growth and biochemical changes of *Vigna mungo* L. Hepper

Morphometric parameter and Biochemical contents	Control	Effect of pesticide in experimental plant											
		Monochrotophos is alone (ml/ 250g of soil)				Monochrotophos with *Pseudomonas aeruginosa* (ml/250g of soil)				Monochrotophos with *Trichoderma viride* (ml/250g of soil)			
		0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
Germinating ability (%)	100 ±5.0	73 ±4.0	66 ±6.0	50 ±8.0	28 ±5.0	100 ±7.0	83 ±5.0	66 ±5.0	66 ±6.0	83 ±5.0	66 ±4.0	50 ±4.0	33 ±2.0
Shoot length (cm)	15 ±0.8	9 ±0.5	9 ±0.3	7 ±0.3	6 ±0.1	11 ±0.5	10 ±0.2	9 ±0.5	9 ±0.1	10 ±0.5	10 ±0.3	9 ±0.5	8 ±0.1
Root length (cm)	8 ±0.1	7 ±0.2	6 ±0.5	5 ±0.2	4 ±0.5	10 ±0.5	10 ±0.1	9 ±0.2	8 ±0.5	9 ±0.8	9 ±0.7	8 ±0.5	8 ±0.1
Chlorophyll A (mg/g fw^{-1})	0.36 ±0.09	0.20 ±0.08	0.17 ±0.03	0.14 ±0.07	0.13 ±0.02	0.28 ±0.09	0.24 ±0.03	0.21 ±0.08	0.19 ±0.05	0.19 ±0.05	0.17 ±0.08	0.15 ±0.09	0.14 ±0.13
Chlorophyll B (mg/g fw^{-1})	0.69 ±0.01	0.24 ±0.09	0.20 ±0.05	0.18 ±0.06	0.15 ±0.08	0.30 ±0.07	0.28 ±0.06	0.23 ±0.03	0.21 ±0.13	0.27 ±0.07	0.25 ±0.09	0.20 ±0.04	0.18 ±0.07
Total Chlorophyll (mg/g fw^{-1})	1.05 ±0.10	0.44 ±0.17	0.37 ±0.08	0.32 ±0.13	0.28 ±0.10	0.58 ±0.16	0.52 ±0.09	0.44 ±0.11	0.40 ±0.18	0.46 ±0.12	0.42 ±0.17	0.35 ±0.13	0.32 ±0.20
Protein (mg/g)	0.35 ±0.08	0.16 ±0.06	0.14 ±0.05	0.13 ±0.03	0.11 ±0.04	0.28 ±0.03	0.26 ±0.08	0.23 ±0.05	0.19 ±0.05	0.21 ±0.08	0.20 ±0.05	0.18 ±0.05	0.16 ±0.06
Carotenoids Mg/plant	0.76 ±0.08	0.43 ±0.07	0.35 ±0.07	0.20 ±0.03	0.12 ±0.03	0.75 ±0.06	0.60 ±0.08	0.50 ±0.05	0.30 ±0.06	0.59 ±0.04	0.50 ±0.08	0.35 ±0.04	0.30 ±0.05

Both *Pseudomonas aeruginosa* and *Trichoderma viridae* moderately degraded the monochrotophos. Chlorophyll, protein and carotenoid contents were decreased in pesticide contained soil when compared with control. All the parameters came to near normal in the pesticide contained soil with microbes (Tables1and 2). Maximum growth was noted in methyl parathion contained soil with both organisms. Maximum growth

Table 2. Degradation of methyl parathion by *Pseudomonas aeruginosa* and *Trichoderma viridae* and their effect on growth and biochemical changes of *Vigna mungo* L. Hepper

Morphometric parameter and Biochemical contents	Control	Effect of pesticide in experimental plant											
		Methyl -Parathion is alone (ml/ 250g of soil)				Methyl -Parathion with *Pseudomonas aeruginosa* (ml/250g of soil)				Methyl -Parathion with *Trichoderma viride* (ml/250g of soil)			
		0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
Germinating ability (%)	100 ±5.0	80 ±8.0	65 ±7.0	45 ±8.0	33 ±5.0	100 ±9.0	100 ±5.0	83 ±5.0	66 ±6.0	100 ±9.0	83 ±7.0	66 ±5.0	50 ±5.0
Shoot length (cm)	15 ±0.8	12 ±0.5	12 ±0.3	9 ±0.2	8 ±0.1	14 ±0.5	14 ±0.3	13 ±0.5	13 ±0.3	14 ±0.8	14 ±0.3	13 ±0.5	13 ±0.2
Root length (cm)	8 ±0.1	7 ±0.8	7 ±0.3	6 ±0.5	6 ±0.1	14 ±0.1	14 ±0.1	13 ±0.3	12 ±0.9	14 ±0.9	14 ±0.5	12 ±0.1	12 ±0.2
Chlorophyll A (mg/g fw^{-1})	0.36 ±0.09	0.23 ±0.05	0.21 ±0.07	0.19 ±0.06	0.15 ±0.07	0.34 ±0.06	0.32 ±0.05	0.31 ±0.06	0.29 ±0.07	0.31 ±0.07	0.30 ±0.07	0.29 ±0.05	0.28 ±0.08
Chlorophyll B (mg/g fw^{-1})	0.69 ±0.01	0.25 ±0.04	0.24 ±0.05	0.21 ±0.05	0.18 ±0.07	0.48 ±0.06	0.44 ±0.06	0.40 ±0.07	0.38 ±0.06	0.35 ±0.14	0.32 ±0.05	0.31 ±0.03	0.29 ±0.07
Total Chlorophyll (mg/g fw^{-1})	1.05 ±0.10	0.48 ±0.09	0.45 ±0.12	0.40 ±0.11	0.33 ±0.14	0.83 ±0.12	0.76 ±0.11	0.72 ±0.13	0.67 ±0.13	0.66 ±0.21	0.62 ±0.12	0.60 ±0.08	0.57 ±0.15
Protein (mg/g)	0.35 ±0.08	0.25 ±0.09	0.22 ±0.05	0.18 ±0.07	0.14 ±0.05	0.30 ±0.05	0.28 ±0.08	0.25 ±0.05	0.21 ±0.08	0.30 ±0.08	0.27 ±0.06	0.24 ±0.05	0.20 ±0.07
Carotenoids Mg/plant	0.76 ±0.08	0.50 ±0.07	0.35 ±0.05	0.21 ±0.07	0.13 ±0.06	0.76 ±0.07	0.70 ±0.08	0.50 ±0.05	0.35 ±0.07	0.60 ±0.05	0.59 ±0.07	0.30 ±0.03	0.30 ±0.07

indicated highest degradation of pesticides. Rani and Lalitha Kumari, (1994) reported that *Pseudomonas putida* degraded methyl parathion. They found that *P.putida* could hydrolyze methyl parathion and use p-nitrophenol as sole carbon source. Kullman and Matsumara, (1996) reported the degradation of β-endosulfan by *Trichoderma harzianum*. Cui zhongli *et al.* (2001) reported that *Plesiomonas* sp. strain M6 was able to hydrolyze methyl parathion to *p*-nitrophenol. A novel organophosphate hydrolase gene designated *mpd* was selected from its genomic library prepared by shotgun cloning. Tejomyee *et al*. (2009) reported that soil fungi capable of degrading monocrotophos (MCP) were isolated from various geographical and ecological sites. Twenty-five strains were isolated by an enrichment method using MCP as a carbon and phosphorus source. On the basis of MCP tolerance capacity exhibited in gradient agar plate assay the isolate M-4, identified as *Aspergillus oryzae* ARIFCC 1054, was selected.

CONCLUSION

The present investigation indicated that the *Pseudomonas aeruginosa* degraded the methyl parathion effectively. This microbial consortiums can be effectively used to degrade methyl parathion from contaminated soils, sediments and waste waters.

Acknowledgements

Authors are thankful to Department of Biotechnology, Thanthai Hans Roever College, Perambalur for providing financial assistance through Project.

REFERENCES

Adhya, TK., Barik S. and Sethunathan, N. 1981. Hydrolysis of selected organophosphorus insecticides by two bacteria isolated from flooded soil. *J. Appl. Bacteriol*., 50: 167-172.

Anonymous. 1997. Monocrotophos. *Pesticide News.,* 38: 6–7.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris Plant Physiol*., 2: 1-15.

Bhadbhade, B.J., Sarnaik, S.S. and Kanekar, P.P. 2002. Biomineralization of an organophosphorus pesticide (Monocrotophos) by soil bacteria. *Journal of Applied Microbiology,* 93: 224–234.

Bhalerao, T.S. and Puranik, P.R. 2007. Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. *International Biodeterioration and Biodegradation*, 59: 315–319.

Cui zhongli, LI., Shunpeng, and Fu Guoping. 2001. Isolation of Methyl Parathion-Degrading Strain M6 and Cloning of the Methyl Parathion Hydrolase Gene. *Applied and environmental microbiology*, 67(10): 4922–4925.

Hewitt, H.G. 1998. *Fungicides in Crop Protection*. CAB International, UK.

IPCS. 1993. *Monocrotophos Health and Safety Guide* No. 80. UNEP and ILO, WHO, Geneva.

Kirk, J.T.O. and R.L. Allen. 1965. Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. Biochem. Biophysics Res. *J. Canada*, 27: 523-530.

Kullman, S.W. and Matsumura, F. 1996**.** Metabolic pathways utilized by Phanerochaete chrysosporium for degradation of the cyclodiene pesticide endosulfan. *Appl. Environ. Microbiol.,* 62: 593-600.

Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.,* 193(1):265-275.

Rani, N.L. and Lalitha Kumari, D. 1994. Degradation of methyl parathion by Pseudomonas putida. *Can. J. Microbial.,* 40:1000-1006.

Singh, S. and Singh, D.K. 2003. Utilization of monocrotophos as phosphorus source by Pseudomonas aeruginosa F10B and Clavibacter michiganense sub sp. Inisidiosum SBL 11. *Canadian Journal of Microbiology,* 49: 101–109.

Skripsky,T. and Loosli, R. 1994. Toxicology of monocrotophos. *Review of Environmental Contamination and Toxicology,* 139:13–39.

Tejomyee, S. Bhalerao, Pravin, and Puranik, R. 2009. Microbial degradation of monocrotophos by *Aspergillus oryzae*. *International Biodeterior,* 63:503-508.

Tomlin, C. (Ed.). 1994. *The Pesticide Manual*, 10th ed. British Crop Protection Council and Royal Society of Chemistry.

Vig, K., Singh, D.K., Agarwal, H.C., Dhawan, A.K. and Dureja, P. 2001. Insecticide residues in cotton crop soil. *Journal of Environmental Science and Health, B*36: 421–434.

****