Research Journal of

Environmental Toxicology

ISSN 1819-3420

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 4 (4): 256-262, 2010
ISSN 1819-3420 / DOI: 10.3923/rjet.2007.124.130

Biodegradation of Pesticidal Residue Using Traditional Plants with Medicinal Properties and *Trichoderma* *

H.D. Subashini, S. Sekar, V.R. Sreelekha Devi,
A. Rajam and S. Malarvannan
M.S. Swaminathan Research Foundation, III Cross Road,
Taramani Institutional Area, Chennai, 600113, India

Abstract: The indiscriminate use of pesticides to control major pests and diseases has led to several adverse consequences and deepened agrarian distress, polluted the environment and created a heavy disturbance in the ecosystem. Ultimately the end users (human and animal beings) are also severely affected. Hence, it is essential to remove these chemo-pollutants from the environment. Biological removal of chemo-pollutants becomes the safest and easiest method. In the present study, some of the traditional plants with medicinal properties viz., *Cipadessa baccifera*, *Clausena dentata*, *Dodonaea angustifolia* and *Melia dubia* along with *Trichoderma viride* were tested for their capacity to degrade the commonly used pesticides namely, endosulfan, acephate and quinalphos under *in vitro* conditions. The pH, nutritional status and the microflora (bacteria and fungal population) of the soil were analysed from zero to 60th day at 20 days interval. The residues of these pesticides detected by gas chromatography revealed that the above mentioned plants along with *Trichoderma* were highly efficient in degrading the pesticides. Improvement in the soil health condition (microbial community) is also proved that the biological method of degradation is safe to the ecosystem.

Key words: Pesticides, medicinal plants, *Cipadessa*, *Clausena*, *Dodonaea*, *Melia*

INTRODUCTION

In India, on an average, 33% of crop loss occurs due to pests and diseases (Puri *et al*., 1999) and runs to an estimated amount Rs. 200 billion (Singh, 1999). Pesticide is an essential ally in the farmers' struggle to protect their crops. Pesticide consumption in India is 288 g ha^{-1}, which is low compared with a global average of 900 g ha^{-1} (Agnihotri, 2000). Forty percent of all pesticides used in India belong to the organochlorine, while 30% belong to the organophosphate category. However, continual and liberal use of pesticides has led to several disturbing consequences on agro-ecosystems and human health (Gupta, 1989; Gunning *et al*., 1992; Mathur, 1998). The incidence of cancer, asthma and diseases of kidney, skin and digestive tract has increased by 20-25% in Punjab. Youngsters at the age of 25-30 are suffering from heart ailments and male infertility (www.punjabilok.com). Some of them, specifically, endosulfan is extremely toxic to fish and aquatic invertebrates (Goebel *et al*., 1982).

Degradation of such harmful pesticides is the need of the hour and there has been enormous studies conducted towards the same. The potential of microorganisms to degrade and remove pesticides from soils has also been successfully attempted (Doris *et al*., 1990; Karanth, 1992). The hydrolysis of organophosphorus insecticide by bacteria in the flooded soil (Adhya *et al*., 1981) and the microbial cleavage for degradation has also been proved (Rosenberg and Alexander, 1979). Liu *et al*. (2001) have isolated *Aspergillus niger*, which was able to degrade Dimethoate. Tu (1992) observed degradation

Corresponding Author: H.D. Subashini, M.S. Swaminathan Research Foundation, III Cross Road, Taramani Institutional Area, Chennai, 600113, India
Tel: 91-44-22541229 Fax: 91-44-22541319

**Originally Published in Research Journal of Environmental Toxicology, 2007*

of haloxyfop, tridiphane and pyroxyfur by soil microbes. Park *et al*. (2006) had achieved the removal of 2,4-DCP from the soil within 10 min when treated with peroxide and minced *Capsella bursa-pastoris* root. *Typha latifolia* commonly known as cattails also serve as a good candidate for removal of methyl parathion from the contaminated water. Lemmon and Pylypiw (1992) studied the persistence of chlorpyrifos, diazinon, isofenphos and pendimethalin after composting with grass clippings. Muller and Korte (1975 and 1976) found that, 12% of the initial aldrin, 3% of the dieldrin and less than 15% of monolinuron and imugan added to municipal solid waste and biosolids feedstock was degraded after composting. Wagner and Zablotowicz (1997) reported that the ryegrass, rice straw and hairy vetch residues were effective in enhancing fluometuron degradation in soils since these amendments were able to enhance the biodegrading potential of indigenous soil microbial population. Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects and they can uptake and convert chemicals quickly to less toxic metabolites in some cases (Schnoor, 1997) and some plants are more effective than others in the remediation of pesticide-contaminated soil and water (Karthikeyan *et al.,* 2004).

However, use of traditional plants in the breakdown of pesticide molecules has received less attention and has been neglected for various reasons. It is highly essential to tap the unexplored area and blend the traditional knowledge with frontier science.

Hence, the present study was aimed to utilize less explored traditional plants along with a fungus, *Trichoderma,* which can accelerate the degrading capacity in different combinations. The efficiency in the pesticide degradation is observed in terms of soil health (physical, chemical and biological characteristics) and the pesticide residue in the soil.

MATERIALS AND METHODS

Plant Material

The leaves of *Cipadessa baccifera*, *Clausena dentata*, *Dodonaea angustifolia* and *Melia dubia* collected from Kolli Hills, Tamil Nadu, were air dried and powdered. *Trichoderma viride* was maintained in the lab at MSSRF.

Treatment

The experiment was conducted at M.S.S waminathan Research Foundation during 2005-2006. The pesticides viz., endosulfan 35 EC (5.7 μL), acephate 75 SP (0.3 μg) and quinalphos 25 EC (8 μL) were amended kg^{-1} of soil. *Trichoderma viride* (10 g) was added along with the shade dried and powdered leaves (6 g) of *Cipadessa baccifera*, *Clausena dentata*, *Dodonaea angustifolia* and *Melia dubia*. The combinations are as follows:

T1 -soil; T2-T1 + pesticides; T3-T1 +*Trichoderma viride*; T4-T2 + *T. viride*; T5-T1+ *Cipadessa baccifera*; T6- T2 + *C. baccifera*; T7-T3 + *C. baccifera*; T8- T4 + *C. baccifera*; T9-T1+ *Clausena dentata*; T10 -T2 + *C. dentata*; T11- T3 + *C. dentata*; T12- T4 + *C. dentata*; T13 -T1 + *Dodonaea angustifolia*; T14- T2 + *D. angustifolia*; T15- T3 + *D. angustifolia*; T16 - T4 +*D. angustifolia*; T17-T1 + *Melia dubia*; T18 - T2 + *M. dubia*; T19 -T3 + *M. dubia*; T20-T4 + *M. dubia*

Moisture at 20-30% was maintained throughout the experiment.

Soil Characteristics

pH, nitrogen and the organic carbon content [wet digestion method (Heanes, 1984)] of the soil were analyzed at 0th, 20th, 40th and 60th days.

GC Analysis of Pesticidal Residue

The soil sample (pesticide amended only - T2, T4, T6, T8, T10, T12, T14, T16, T18 and T 20) was extracted with DCM and the organic phase was dried with $MgSO_4$. The metabolites were then diluted with hexane to yield a 20% hexane-DCM solution, which was applied to a silica gel column. Endosulfan, acephate, quinalphos were added as an internal standards, the residues were then analyzed for GC using FID (Ki-Souk Nam and Jerry King, 1994).

Microbial Population

The soil sample was tested for the presence of microbes at different treatments on 0th and 60th day. One gram of soil sample was serially diluted and 0.1 mL (10^{-5}) was plated on the Nutrient agar for bacteria and Potato dextrose agar for fungi at 10^{-4} concentrations. The THC colonies (bacteria) and Cfu (fungi) were counted.

RESULTS AND DISCUSSION

Physico-chemical Characteristics of the Treatments

The soil samples were analysed at 0th, 20th, 40th and 60th days and their results are given below.

Physical Characteristics

From the results, irrespective of the treatments and duration, the pH was in the range of 9-10 with moisture of 20-30%, which would have enhanced the microbial population and played a major role in pesticide degradation. Gi-Seok *et al*. (2002) have proved that at alkaline pH, the bacteria were able to degrade the organochlorine. Each pesticide has specific characteristic to degrade at specific pH and moisture content (Singh *et al*., 2003). Pradyna *et al*. (2004) reviewed the enzymatic hydrolysis of few pesticides in the pH range of 7.5 to 9.5 and at a temperature 35-40°C.

Chemical Characteristics

Organic carbon and available nitrogen were analyzed at 20 days interval. A considerable increase in the organic carbon was observed in T16 (soil + pesticide + *T. viride* + *D. angustifolia*) followed by T8 (soil + pesticide + *T. viride*) after 20 days of observation. In general, there was a slight increase in the organic carbon content with the increase in the days of exposure. However, it decreased in the 60th day. It was a different trend in the case of the available nitrogen. It increased from 58 to 73% in T10 and consistent in T16 (78-77%) in the first two duration and later increased to 83. Contrastingly, there was a decrease in the content in T18 and T 20, which was similar to control (T2). Application of green manure along with the plants waste, improved the Organic Carbon (OC) content and available N, P and K in the soil (Vinod Kumar *et al*., 1999). The additions of manure can double the soil carbon or nitrogen levels (Jenkinson *et al*., 1994; Powlson, 1994).

Pesticidal Residue-GC Analysis

The combination of these medicinal plants and the beneficial fungus has drastically reduced the acephate content in the soil. The results from GC revealed that the amount of acephate was reduced from 2.09 to 0.74 μg mL^{-1} in soil containing *D. angustifolia* as a substrate, followed by *Cipadessa baccifera* (from 1.69 to 0.82 μL g^{-1}), *Melia dubia* (from 1.98 to 0.79 μL g^{-1}) and *T. viride* (1.88 to 0.86 μg mL^{-1}) alone. The combination of *M. dubia* and *T. viride* has efficiently reduced the acephate concentration up to 0.17 μg mL^{-1} from the soil (Fig. 1). The acephate concentration was constant in T2 (soil + pesticide) at 0th and the 60th day (1.90 and 1.77 μg mL^{-1}), this proves that the plants and *T. viride* have major role in degradation of acephate in the soil (Fig. 1).

The reduction of endosulfan residue from the soil was efficient when *T. viride* in combination with different medicinal plants were used. Non-detectable amount of endosulfan observed on the 60th day in the soil treated with *T. viride* (T4), *C. baccifera* (T6) and *M. dubia* (T 20). *Clausena dentata* in

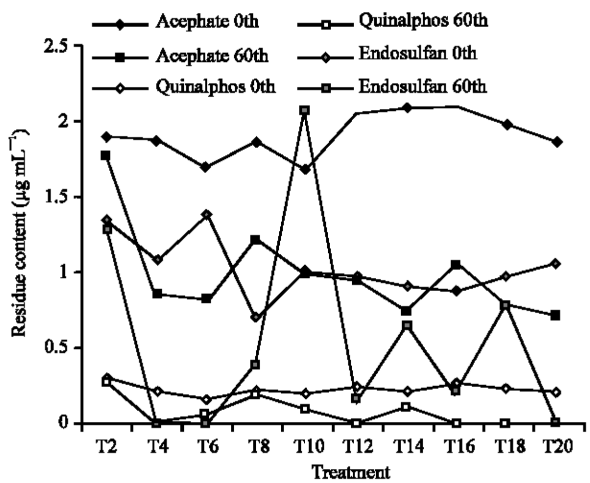

Fig. 1: Pesticide residue in the soil treated with plants and *Trichoderma*

Table 1: Enumeration of microbial population (bacteria and fungi) in the pesticide amended soils treated with traditional plants and *Trichoderma*

	Bacteria (THC×10^5)				Fungi (Cfu×10^4)			
Treatment	0th	20th	40th	60th	0th	20th	40th	60th
T1	40.33	60.67	43.67	37.33	34.33	23.67	18.00	13.00
T2	35.67	72.00	23.67	62.33	25.00	15.67	23.00	16.33
T3	64.00	45.33	30.33	76.67	14.67	18.67	23.67	26.67
T4	42.00	42.67	39.00	63.00	20.67	20.00	22.00	24.33
T5	86.00	131.33	120.00	178.67	18.33	21.33	24.00	18.00
T6	217.00	199.33	184.00	186.33	21.33	18.67	17.67	20.67
T7	140.67	139.00	145.33	181.67	14.00	18.33	12.67	17.00
T8	178.33	174.33	184.67	273.33	18.00	19.67	25.33	23.00
T9	193.67	123.00	127.00	260.33	26.33	16.67	16.00	32.33
T10	248.33	159.33	129.33	177.00	17.00	18.00	26.00	20.00
T11	116.00	93.00	166.67	192.33	13.67	23.33	12.67	13.33
T12	87.00	83.33	85.33	140.00	28.33	17.67	19.00	14.33
T13	71.67	71.67	93.00	141.33	24.33	16.33	20.67	23.33
T14	110.67	84.00	134.33	156.33	24.33	12.33	25.00	28.00
T15	75.33	90.00	182.33	231.33	16.66	21.33	15.67	20.67
T16	93.33	87.33	96.00	114.67	12.33	26.00	26.67	23.67
T17	116.67	158.00	150.67	155.67	19.33	26.00	23.33	31.67
T18	79.00	92.67	91.33	164.67	14.33	35.33	31.33	31.67
T19	75.67	59.67	96.67	133.33	10.00	31.00	27.00	27.00
T20	67.00	63.67	112.67	134.67	15.00	31.00	25.70	25.00
CD(p = 0.05)	17.53	23.68	23.07	20.50	4.55	5.47	5.82	5.53

Each value mean of triplicate; THC: Total Heterotropic Count; Cfu: Colony Forming Units

combination was able to reduce to 0.16 µg mL^{-1} where as in untreated soil it was 1.30 µg mL^{-1}. *Dodonaea angustifolia* in combination could reduce up to 0.20 µg mL^{-1} and *Cipadessa baccifera* could reduce up to 0.39 µg mL^{-1} (Fig. 1). Smith (1995) reported the degradation of organochlorine pesticide using *T. viride*. The initial amount of azinphos methyl was reduced to non-detectable levels in the presence of plant, alfalfa (Flocco *et al*., 2006).

Non-detectable level of quinalphos was recorded in all the treatments (T4, T12, T16, T18 and T20) with *T. viride*. In other treatments, *C. baccifera*, *C. dentata*, *D.angustifolia* were able to decrease the residue content of quinalphos up to 0.06, 0.09 and 0.11 µg mL^{-1} (Fig. 1). Azuma *et al*. (1994) reported the removal of diazinon, fenitrothion and chlorpyrifos, captan, isoprothiolane and chlorothalonil and simazine with the help of the mung beans from wastewater. Sun *et al*. (2004) had investigated the degradation of aldicarb, an oxime carbamate insecticide, in the soil grown with corn, mung bean and cowpea and reported that such degradation was mainly due to plant-promoted degradation in the rhizosphere.

In general, untreated pesticide amended soil (T2) showed the same amount of pesticides (acephate, quinalphos and endosulfan) both on zero and 60th day.

Microbial Population

The growth of the bacteria gradually increased from the zero day to the 60th day (Table 1). The growth was higher in the soils inoculated with the plants; *Clausena* and *Cipadessa* enhanced the growth of the bacteria when compared to the other two plants. *T. viride* did not support the growth of bacteria as efficient as the plants. Similar work was done by Siciliano and Germida (1997), they have proved that bacterial inoculants of forage grasses enhanced the degradation of 2-chlorobenzoic acid in soil. Pradyna *et al*. (2004) reviewed the biodegradation of organophosphorus pesticides in the presence of microbes. *Dodonaea* and *Melia* in the treatments (T16, T17, T18, T19, T20) have drastically enhanced the fungal population from 20th day onwards. In the other treatments (T4, T5, T6, T7) with *Clausena* and *Cipadessa*, not much difference in the fungal population was recorded (Table 1). Degradation of pesticide by white rot fungi was reported by Gary *et al*. (2002). De Schrijver and De Mot (1999) isolated actinomycetes capable of degrading various pesticides. Aislabbie and Lloyd (1995) reviewed the bacterial degradation of pesticide.

CONCLUSION

The present study revealed the abilities of the medicinal plants to aid in the removal of pesticides from the pesticide-contaminated soil. However the medicinal plants enhance the growth of microbes, which in turn helps in the detoxification of pesticides. The medicinal plants aid as manure to the soil in maintaining the soil pH, carbon and the nitrogen contents. This is preferred over other techniques because of its access, availability, low-cost and ecofriendly nature.

REFERENCES

Adhya, T.K., S. Barik and N. Sethunathan, 1981. Hydrolysis of selected organophosphorus insecticide by two bacteria isolated from flood soil. J. Applied Bacteriol., 50: 167-172.

Agnihotri, N.P., 2000. Pesticide consumption in agriculture in India-An update. Pesticide Res. J., 12: 150-155.

Aislabbie, J. and G.J. Lloyd, 1995. A review of bacterial degradation of pesticides. Aust. J. Soil Res., 33: 925-942.

Azuma, T., M. Niiro and H. Motobu, 1994. Removal of pesticides from wastewater at golf courses using plants (removal by mung beans; *Phaseolus radiatus* L). Biomed. Mater. Eng., 4: 127-137.

De Schrijver, A. and R. De Mot, 1999. Degradation of pesticides by actinomycetes. Crit. Rev. Microbiol., 25: 85-119.

Doris, M.S., N. Ramesha and N.G.K. Karanth, 1990. Insecticide Contamination in Groundnuts and Biological Methods for Cleaning. Shetty, H.S. and H.S. Prakash, (Eds.), Proc. Adv. Seed Sci. Technol. (Mysore, India), pp: 368-371.

Flocco, C.G., M.P. Carranza, L.G. Carvajal, R.M. Loewy and A.M. Pechen de 'Angelo, 2006. Removal of azinphos methyl by alfalfa plants (*Medicago sativa* L.) in a soil-free system. Chemosphere, 63: 1124-1129.

Gary, D.B., M. Friloux and A. Walker, 2002. Degradation of contrasting pesticides by white rot fungi and its relationship with lignolytic potential. FEMS Microbiol. Lett., 212: 59-63.

Gi-Seok Kwon, J.E. Kim, T.K. Kim, H.Y. Sohn, S.C. Koh, K.S. Shin and D.G. Kim, 2002. *Klebsiella pneumoniae* KE-1 degrades endosulfan without formation of the toxic metabolite, endosulfan sulfate. FEMS Microbiol. Lett., 215: 255-259.

Goebel, H.S., Gorbach, W. Knauf, R.H. Rimpau and H. Huttenbach, 1982. Properties, effects, residues and analytics of the insecticide endosulfan. Residue Rev., 83: 40-41.

Gunning, R.V., M.E. Balfe and C.S. Easton, 1992. Carbamate resistance in *Helicoverpa armigera* in Australia. J. Aust. Entomol. Soc., 31: 97-103.

Gupta, Y.P., 1989. Pesticide Poisoning. Sci. Rev., 26: 491-492.

Heanes, D.L., 1984. Determination of total organic C in soil by an improved chromic acid digestion and spectrophotometric procedure. Commun. Soil Sci. Plan, 15: 1191-1213.

Jenkinson, D.S., N.J. Bradbury and K. Coleman, 1994. How the Rothamsted Classical Experiments Have Been Used to Develop and Test Models for the Turnover of Carbon and Nitrogen in Soil. In: Long-Term Experiments in Agricultural and Ecological Sciences. Leigh, R.A. and A. Johntson (Eds.), E.CAB Int., Wallingford, UK., pp: 117-138.

Karanth, N.G.K., 1992. Abatement of Pesticide Residues Through Biodegradation. In: Environment and Biodegradation. Agrawal, V.P. and S.V.S. Rana (Eds.), Society of Biosciences, India, pp: 243-252.

Karthikeyan, R. Lawrence Davis, Larry Erickson, Kassim Al-Khatib, Peter Kulakow, Philip Barnes, Stacy Hutchinson and Asil Nurzhanova, 2004. Potential for plant-based remediation of pesticide-contaminated soil and water using nontarget plants such as trees, shrubs and grasses. Crit. Rev. Plant Sci., 23.

Ki-Souk Nam and Jerry W. King, 1994. Coupled SFE/SFC/GC for the trace analysis of pesticide residues in fatty food samples. J. High Resolution Chromatog., 17: 577-582.

Liu, Y., Ying-Cheng Chung and Ya Xiong, 2001. Purification and characterization of a dimethoate-degrading enzyme of *Aspergillus niger* ZHY256, isolated from sewage. Applied Environ. Microbiol., 67: 3746-3749.

Lemmon, C.R. and H.M. Pylypiw, 1992. Degradation of diazinon, chlorpyrifos, isofenphos and pendimethalin in grass and compost. Bull. Environ. Contamin. Toxicol., 48: 409-415.

Mathur, S.C., 1998. Pesticide industry in India. Pest. Inform., 23: 17-19.

Muller, W.P. and F. Korte, 1975. Microbial degradation of benzo-(a)-pyrene, monolinuron and dieldrin in waste composting. Chemosphere, 4: 195-198.

Muller, W.P. and F. Korte, 1976. Ecological Chemical Evaluation of Waste Treatment Procedures. In: Environmental Quality and Safety: Global Aspects of Chemistry, Toxicology and Technology as Applied to the Environment. Coulston, F. and F. Korte (Eds.), Academic Press, New York, pp: 215-236.

Park, J.W., B.K. Park and J.E. Kim, 2006. Remediation of soil contaminated with 2,4-dichlorophenol by treatment of minced shepherd's purse roots. Arch. Environ. Contam. Toxicol., 50: 191-195.

Powlson, D.S., 1994. Quantification of Nutrient Cycles Using Long- Term Experiments. In: Long-Term Experiments in Agricultural and Ecological Sciences. Leigh, R.A. and A.E. Johnston (Eds.), CAB Int., Wallingford, UK., pp: 97-115.

Pradyna, P.K., J.B. Bharati, M.D. Neelima and S.S. Seema, 2004. Biodegradation of organophosphorus pesticide. Prod. Ind. Natn. Sci. Aca., 70: 57-70.

Puri, S.N., K.S. Murthy and O.P. Sharma, 1999. Pest Problems in India-Current Status. Indian J. Plant Protection, 27: 20-31.

Rosenberg, A. and M. Alexander, 1979. Microbial cleavage of various organophosphorus insecticide. Applied Environ. Microbiol., 37: 886-891.

Schnoor, J.L., 1997. Phytoremediation. Technology Evaluation Report. Ground-Water Remediation Technologies Analysis Center, Pittsburgh, pp: 1-43.

Singh, S.P., 1999. Biological control in India. Indian J. Plant Prot., 27: 126-138.

Singh, B.K., A. Walker, J.A.W. Morgan and D.J. Wright, 2003. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Applied Environ. Microbiol., 69: 5198-5206.

Siciliano, S.D. and J.J. Germida, 1997. Bacterial inoculants of forage grasses that enhance degradation of 2-Chlorobenzoic acid in soil. Environ. Toxicol. Chem., 16: 1098-1104.

Smith, W.H., 1995. Forest occurrence of *Trichoderma* sp. emphasis on potential organochlorine (xenobiotic) degradation. Ecotoxicol. Environ. Safety, 32: 179-183.

Sun, H., J. Xu, S. Yang, G. Liu and S. Dai, 2004. Plant uptake of aldicarb from contaminated soil and its enhanced degradation in the rhizosphere. Chemosphere, 54: 569-574.

Tu, C.M., 1992. Effect of three newer pesticides on microbial and enzymatic activities in soil. Bull. Environ. Contam. Toxicol., 49: 120-128.

Vinod Kumar, B.C. Ghosh and R. Bhat, 1999. Recycling of crop wastes and green manure and their impact on yield and nutrient uptake of wetland rice. J. Agric. Sci., 132: 149-154.

Wagner, S.C. and R.M. Zablotowicz, 1997. Utilization of Plant Material for Remediation of Herbicide-contaminated Soils. In: Phytoremediation of Soil and Water Contaminants. Anderson, T.A., E.L. Kruger and Joel R. Coats (Eds.), American Chemical Society, Washington, DC., pp: 65-76.

www.punjabilok.com/agriculture/medicos_workshop.htm. Impact of pesticides on health: A critical and in-depth scientific analysis.