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Research Article

Phytoremediation of λ-cyhalothrin pesticidal residue using traditional plants with medicinal properties and also by using the fungus *Trichoderma* species

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Abstract

Pesticides are indispensable to modern agriculture. Currently among the various groups of pesticides that are being used world over, organophosphates from a major and most widely used group accounting for more than 36% of the total world market. 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. Considering the toxic effects of pesticides, it is essential to remove these chemo-pollutants from the environment. Biological removal of chemo-pollutants becomes the method of choice since micro-organisms can use a variety of xenobiotic compounds including pesticides for their growth and mineralize and detoxify them. 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 take steps 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 dentate, Dodonaea angustifolia* and *Melia dubia* along with *Trichoderma viride* were tested for their capacity to degrade the commonly used pesticide namely, λ-cyhalothrin under *in vitro* conditions. The pH, nutritional status and the microflora (bacteria and fungal population) of the soil were analyzed 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) also proved that the biological method of degradation is safe to the ecosystem.

Keywords: Pesticides, Medicinal plants, *Cipadessa, Clausena, Dodonaea, Melia*

Introduction

Nowadays, one of the major problems facing the industrialized world is the contamination of soils, groundwater and sediments with pesitcides. Despite the valuable contribution associated with the use of pesticides, many of these biologically active chemicals represent a potential hazard to humans and to nature (Laura-Dorina Dinu *et al*., 2011). In India, on an average, 33% of crop loss occurs due to pests and diseases (Puri *et al*., 2013) 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 prolonged 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 TamilNadu. Youngsters at the age of 25-30 are suffering from heart ailments and male infertility. Some of them, specifically, endosulfan is extremely toxic to fish and aquatic invertibrates (Goebel *et al*., 2010).

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) Lin *et al*., (2001) have isolated *Aspergillus niger*, which was able to degrade Dimethoate. Tu (1992) observed degradation 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 bursapastoris* 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 (2012) reported that the ryegrass, rice sraw and hairy vetch residues were effective in enhancing flumeturon 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 was 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 dentate*, *Dodonaea angustifolia* and *Melia dubia* collected from Kolli Hills, Tamil Nadu, were air dried and powdered. *Trichoderma viride* was screened from λ-cyhalothrin contaminated soil sample from Pudukkottai district.

Treatment

The experiment was conducted at J.J College of Arts and Science during the month of September and October 2013. The pesticides λ-cyhalothrin 35 EC (5.7 μL), was amended kg^{-1} of soil. *Trichoderma viride* (10ml) was added along with the shade dried and powdered combinations as follows:

T1- soil; T2-T1 + pesticides; T3-T1 + *Trichoderma viride;* T4-T2 + *Trichoderma viride;* T5-T1+

Cipadessa baccifera; T6-T2 + *Cipadessa baccifera*; T7-T3 + *Cipadessa baccifera*; T8-T4 + *Cipadessa baccifera;* T9-T1 + *Clausena dentata;* T10-T2 + *Clausena dentata;* T11-T3 + *Clausena dentata;* T12-T4 + *Clausena dentata;* T13-T1 + *Dodonaea angustifolia;* T14-T2 + *Dodonaea angustifolia;* T15-T3 + *Dodonaea angustifolia;* T16-T4 + *Dodonaea angustifolia;* T17-T1 + *Melia dubia;* T18-T2 + *Melia dubia;* T19-T3 + *Melia dubia;* T20-T4 + *Melia 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 0^{th}, 20^{th}, 40^{th} and 60^{th} day.

GC Analysis of Pesticidal Residue

The soil sample (pesticide amended only – T2, T4, T6, T8, T10, T12, T14, T16, T18 and T20) 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, λ-cyhalothrin was added as an internal standard, and 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 0^{th} and 60^{th} day. One gram of soil sample was serially diluted and 0.1mL (10^{-5}) was plated on the Nutrient agar for bacteria and Potato dextrose agar for fungi at 10^{-1} concentrations. The THC colonies (bacteria) and CFU (fungi) were counted.

Results and Discussion

Physico-Chemical Characteristics of the Treatments

The soil samples were analyzed at 0^{th}, 20^{th}, 40^{th}, and 60^{th} days and their results are given below.

Physical Characteristics

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 35-40ºC. From the results, it was observed that irrespective of the treatments and duration, the pH was in the range of 9-10 with moisture content of 20-30%, which would have enhanced the microbial population and played a major role in pesticide degradation.

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 + *Trichoderma viride* + *Dodonaea angustifolia*) followed by T8 (soil + pesticide + *Trichoderma 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 60^{th} 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 duration and later increased to 83. In contrast, there was a decrease in the content in T18 and T20, 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 sol (Vinod Kumar *et al*., 1999). The addition of manure can double the soil carbon or nitrogen levels (Jenkinson *et al*., 1994; Powlson, 1994)

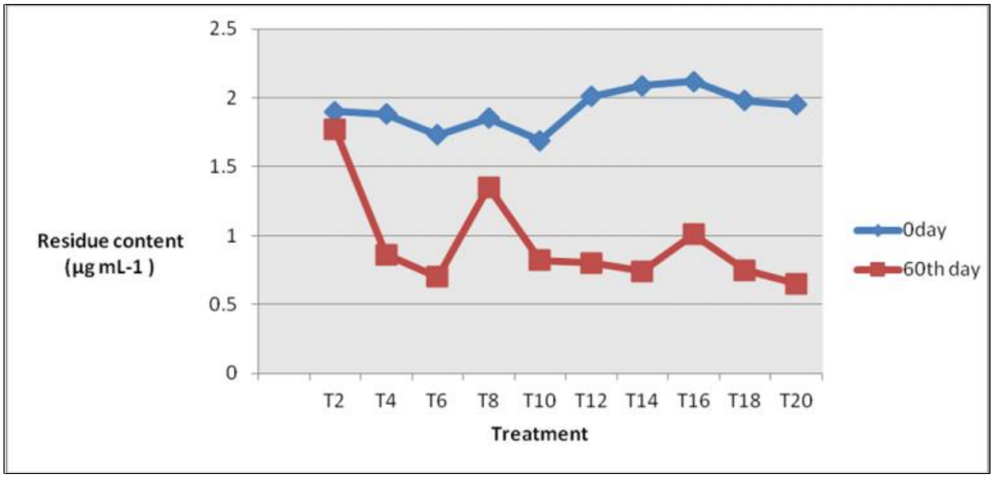
Pesticidal Residue – GC Analysis

The combination of these medicinal plants and the beneficial fungus drastically reduced the λ-cyhalothrin content in the soil. The results from GC revealed that the amount of λ-cyhalothrin was reduced from 2.09 to 0.74 µg mL^{-1} in soil containing *Dodonaea angustifolia* as a substrate,

Table 1. Pesticide residual value on 0 day and 60th day

Treatments	0th day	60th day
T2	1.90	1.77
T4	1.88	0.86
T6	1.73	0.70
T8	1.85	1.35
T10	1.69	0.82
T12	2.01	0.80
T14	2.09	0.74
T16	2.12	1.01
T18	1.98	0.75
T20	1.95	0.65

Figure 1. Pesticide residue in the soil treated with plant leaf powder and *Trichoderma viride*



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 *Trichoderma viride* (1.88 to 0.86 µg mL^{-1}) alone. The combination of *Melia dubia* and *Trichoderma viride* efficiently reduced the λ-cyhalothrin concentration up to 0.17 µg mL^{-1} from the soil (Fig 1). The λ-cyhalothrin 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 *Trichoderma viride* have major role in degradation of λ-cyhalothrin in the soil (Fig1).

Smith (1995) reported the degradation of organochlorine pesticide using *Trichoderma viride*. The initial amount of azinphos methyl was reduced to non-detectable levels in the presence of plant, alfalfa (Flocco et al., 2006). Sun *et al*. (2004) had investigated the degradation of aldicarb, an oxime carbarnate 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 λ-cyhalothrin both on zero and 60th day.

Microbial Population

The growth of the bacteria gradually increased from the zero day to the 60th day (Table 2). 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.

Table 2. Enumeration of microbial population (bacteria and fungi) in the pesticide amended soils treated with traditional plants and *Trichoderma viride*

	Bacteria (THC×10^5)				Fungi (Cfu×10^4)			
Treatment	**0**	**20^{th}**	**40^{th}**	**60^{th}**	**0**	**20^{th}**	**40^{th}**	**60^{th}**
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	49.00	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	148.33	159.33	229.33	277.00	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	124.00	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	101.33	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	23.07	20.50	20.50	4.55	5.47	5.82	5.53

Each value is a mean of triplicate; THC: Total Heterotropic Count; CFU: Colony Forming Units

Trichoderma 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 2). 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. 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 eco-friendly nature.

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