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Influence of Organic Amendments on the Degradation of Endosulfan

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Abstract A laboratory experiment was conducted to study the effect of soil amendments with four different sources of organic matter on the dissipation rate of endosulfan. Farm yard manure (FYM), vermi compost, leaf compost and charcoal, dried, ground (<4 mm) and thoroughly mixed with a calcareous soil at a rate of 2.5 % and placed in plastic pots. Endosulfan was added at 10 µg level. In 30-day incubation period the degradation was fastest in vermin compost, 88.48 %, followed by FYM, seababul leaves and charcoal, recording 87.21 %, 79.41 % and 68.39 %, respectively, as compared to un amended treatment where slow degradation of 65.53 % was observed. The half life was 18.8 days in un-amended soil as compared to 1.01–1.29 days in vermi compost, farm yard manure, charcoal and leaf compost. The amendment with vermin compost, followed by FYM was found to be most effective and enhances the degradation as compared to the other amendments.

Keywords Endosulfan · α and β isomer · Dissipation · Organic amendments

Endosulfan (1,2,3,4,7,7-hexachlorobicyclo-2, 2,1-heptene-2,3-bis-hydroxy methane-5, 6 sulfite) is an organochlorine cyclodiene insecticide used all over the world for the control of various insect pests on variety of food and non crop products. Endosulfan is a mixture of two isomers alpha and beta (Fig. 1) in the ratio 7:3, both are toxic to aquatic animals (Jayshree and Vasudevan 2007). It is non systemic insecticide, and acaricide with contact and stomach action. It is used against a wide range of sucking and chewing insect pests, notably of the orders Lepidoptera, Coleoptera, Heteroptera, Homoptera, Thysanoptera, Diptera, and some species belonging to the order of Acarina. It is used on numerous crops such as cotton, tobacco, vegetables, fruits, corn, cereals, oilseeds, potatoes, tea, and coffee. The technical endosulfan has LD $_{50}$ (Rat)-Oral 160 mg kg^{-1} (male), 22.7 mg kg^{-1} (female); Dermal (Rat) >500 mg kg^{-1}, (Rabbit) 359 mg kg^{-1}. It is classified as a Class I toxic substance and is highly toxic to fish and birds. Endosulfan is the primary cyclodiene insecticide and was widely used in India till 2011; however, it has recently been stopped in certain states of India. Degradation rate of endosulfan in soils is affected by many factors such as soil microorganisms, soil pH, moisture level, and organic matter content (Awashti et al. 2000). It has been reported that the rate of degradation of endosulfan in ordinary soils was higher than in sterile soils (Singh et al. 2000)] Moreover, volatilization is the dominant form in dissipation of endosulfan and its metabolites from the environment (Kathpal et al. 1997) In another study, it was reported that the rate of degradation of endosulfan in soil increased significantly with increasing soil alkalinity, at pH 3 almost no degradation of endosulfan took place, slight degradation at pH 5, significant increase at pH 7.5–8.5, and at pH 10–12 almost all the added endosulfan was rapidly converted to endosulfan diol. The same study reported that soil moisture and aeration affected the degradation rate of endosulfan and the pathways of its metabolism, as its degradation rates decreased significantly in flooded soils compared to non-flooded soils. Organic matter in soil affects soil chemical and physical properties and increase in its level stimulates the growth of soil microflora, which in turn will affect the

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rate of biodegradation of pesticides in soil (Raghed et al. 2004; Awashti et al. 2000). Because of its broad spectrum of activity, endosulfan is popular with the farmers in India and large areas of cultivation use endosulfan either as foliar application or as granular broadcast. Supervised field trials on various crops like vegetables, pulses and oilseeds have revealed that residues of endosulfan are below the Codex Maximum Tolerance limit of 2 mg/kg (Gopal and Mukherjee 1993; Mukherjee and Gopal 1998).Despite judicious use endosulfan has been detected in atmosphere, soil, water, sediment, surface water rain water and food stuffs (Kwon et al. 2002). Endosulfan is hydrophobic and persists in soil for more than 1 year. Endosulfan is degraded by attack on the sulfite group by oxidation and or by hydrolysis to form the toxic endosulfan sulfate and non toxic diol, respectively (Fig. 1), Brar and Aust (1997). The other non toxic metabolites of endosulfan are endosulfan ether and endosulfan lactone. The pesticide residue definition of Codex for setting MRL includes endosulfan –α, endosulfan –β and only the toxic metabolite, endosulfan sulfate. There are reports of degradation of endosulfan by use of microbes (Mukherjee and Gopal 1994; Mukherjee and Mittal 2005). The use of pesticides for agricultural purposes is on the increase because of the need to improve crop production and control of pests, weed infestation and insect outbreaks. The unused pesticides are dumped in land fills which are overflowing with Municipal solid wastes. Easy techniques need to be devised to minimize the contamination in soil and remediation of soil.

The aim of the present work was to evaluate the influence of organic amendments type on the persistence on endosulfan in soil.

Materials and Methods

Endosulfan technical mixture of α and β in the ratio 2:1) (mp 88°C and 98.7 % pure) was obtained gratis from Excel India Ltd (Mumbai, India). It was recrystallized from methanol to obtain the analytical standard. NMR and IR spectroscopy confirmed the identity of the compound. Acetone, dichloromethane and hexane were procured from Merck India Ltd., were glass distilled before use. The other equipments used in the experiment were reciprocal shaker, Remi Centrifuge Table Top, rotary evaporator with vacuum pump. GLC system (Varian CP-3800)—Consisting of a autosampler, injector port PTV1079 and electron capture detector.

Field soil from Indian Agricultural Research Institute (IARI) experimental farms the physicochemical characteristics listed in Table 1 was taken at plough depth (0–15 cm) taken, air dried and sieved. The amendments rice straw, farmyard manure, vermi compost and charcoal were ground passed through 4 mm mesh sieve prior use.

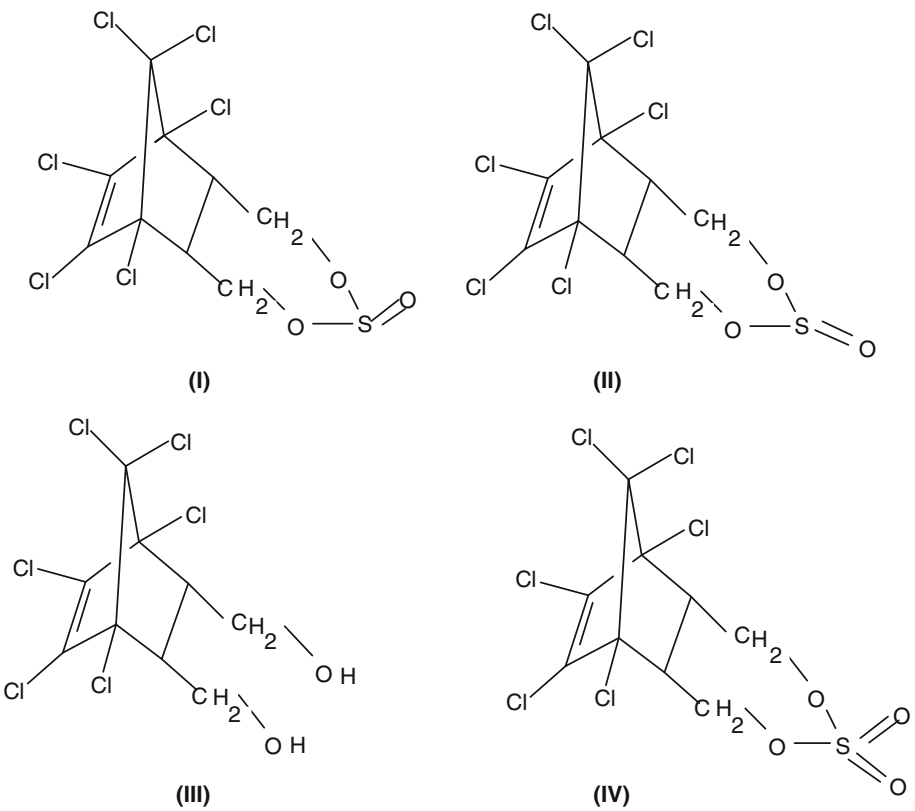

Fig. 1 Structure of α-Endosulfan (I) β-endosulfan (II), endosulfan diol (III) and endosulfan sulfate (IV)

Table 1 Physico-chemical properties of the IARI soil

Properties	Values
Mechanical analysis	
Sand (%)	64.9
Silt (%)	18.0
Clay (%)	17.1
Textural class	Sandy loam
Bulk density (g cm^{-1})	1.45
Field capacity (%)	17.61
Chemical analysis	
pH (soil:water = 1:2.5)	7.41
Electrical conductivity (dsm-1) (soil:water = 1:2.5)	0.35
Cation exchange capacity (Cmol (p^{+}) kg^{-1})	7.35
Organic carbon (%)	0.39

To soil samples (50 g) in glass jars in triplicate were mixed with the amendments at 2.5 % (w/w). An un-amended treatment was set to serve as control. All the treatments were maintained at 13 % field capacity moisture regime and appropriate amount of the amendment was to the and incubated in for 48 h at room temperature 25°C. 5.0 mL of 100 µL/mL of standard solution endosulfan in acetone was added to the wide mouth glass jars and allowed to evaporate. To this soil samples along with the amendments were added. Fifty gram soil samples in triplicate were fortified with endosulfan at 10 µg level. The moisture regime of all the samples was maintained at field capacity levels by periodically weighing and adding appropriate amount of water. These treatments are bio-stimulators for degradation process in soil. A recovery experiment was conducted in triplicate by spiking soil (50 g) at 10 µg level with 5 mL of 100 µL/mL standard solution of endosulfan (α and β in the ratio 2:1). The sample was processed as given below.

Samples were incubated at 25°C. Periodic sampling was carried out on 0, 3, 5, 10, 15, 20, 30, 40, and 60 days. Each set of experiment was carried out in triplicate. Acetone (50 mL) was added to each sample and were shaken using a horizontal shaker for 4 h. The extract was centrifuged on a Remi centrifuge at 200 rpm and filtered. The filtrate was s concentrated to 10 mL, using a rota vapour. The concentrate was transferred to a separatory funnel and 2 % saline water (100 mL) was added and partitioned into dichloromethane (30 mL), passed through anhydrous sodium sulfate and stored. The aqueous portion was partitioned two more times with dichloromethane (2 × 30 mL). The combined dichloromethane phase was concentrated to dryness in a rotary evaporator and subjected to column clean up. The residue was dissolved in 10 mL hexane, and passed over a glass column (30 cm and 1.5 cm ID) packed with silica gel (5 g, 60–120 mesh), sandwiched between layers of anhydrous sodium sulfate (2 g).The column was pre-washed with 50 mL hexane, and the solvent discarded. The organic phase was further cleaned over a column of Florisil. The column eluant was evaporated under reduced pressure and reconstituted in hexane. The samples were analyzed by GLC (Varin CP Sil 3800) using ECD detector.

The metabolite of endosulfan (I), endosulfan diol (III) was prepared in the laboratory to serve as authentic standard sample. Endosulfan (500 mg) was dissolved in ethanol (30 mL) and subjected to alkaline hydrolysis with 10 % potassium hydroxide (15 mL). The reaction mixture was refluxed for 4 h and worked up by neutralizing with dilute hydrochloric acid and further partitioning into diethyl ether thrice (3 × 30 mL). The organic solvent was removed and endosulfan diol was obtained as an oil Rf 0.0726 (75:25 hexane-benzene), IR ν Nujol cm^{-1} 3200 (OH), 1590 (C=C); NMR ($CDCl_3$: δ 3.9 (2H, d, CH2-OH), 3.6 (2H, t, CH-CH2), 3.2 (2H, d, CH2-OH) and 2.35 (2H, bs, OH) Fig. 1. The endosulfan sulfate (IV), the oxon metabolite of endosulfan was prepared by stirring endosulfan (100 mg) dissolved in ethanol with 10 % aqueous solution of potassium permanganate for 4–5 h. The reaction mixture was worked up. The organic solvent evaporated and the compound was subjected to column chromatography over silica gel. The oxon metabolite (IV) of endosulfan was obtained as pale yellow crystalline solid, Rf 0.54 (10 % acetone -benzene), (IR ν KBr cm^{-1}1700 (sul fate group) and 1600 (double bond).; NMR ($CDCl_3$: δ 4.55 (4H, q, 2CH2-O) and 3.5 (2H, bs, 2C-H), 2.21 (t, 3H CH_3), 3.98 (d, 2H, CH_2), (8.20 (s,1 H, Ar–H). This was used as a reference standard for quantification of the presence of the endosulfan diol and the endosulfan sulfate formed during the dissipation experiment.

The analysis was carried out on Varian GLC (model No. CP-3800) fitted with an auto sampler and electron capture detector. The column used was a CP-Sil 5 (30 m × 0.25 mm × 0.25 μ). The column temperature was maintained at 170°C hold for 2 min @ 3°C/min raise to 210°C and @ 30°C/min 260 °C hold for 5 min while the injector port and the detector were set at 250°C and 300°C, respectively. The carrier gas nitrogen flow was maintained at 2 mL min^{-1} and make up flow was 27 mL/min**.** The retention time of α-endosulfan, β-endosulfan and endosulfan sulfate were 8.57, 10.38 and 12.38 min. respectively.

Results and Discussion

The percent recovery of endosulfan (α and β) form spiked samples were 97.8 ± 0.02, 98.2 ± 0.04, 97.9 ± 0.02, 98.7 ± 0.03 and 98.9 ± 0.05, in untreated soil, soil treated

Table 2 Residue of endosulfan isomers in soil amended with different amendment

Amendment	Sampling days	Average residues (mg/kg)			
		α-endosulfan	β endosulfan	Endosulfan sulfate	Total
Vermicompost					
	0	6.804 ± 0.02	2.91 ± 0.02	ND	9.72 ± 0.02
	3	5.526 ± 0.04	2.48 ± 0.03	0.27 ± 0.03	8.28 ± 0.03
	5	4.82 ± 0.02	2.01 ± 0.02	0.40 ± 0.03	7.23 ± 0.02
	7	3.48 ± 0.03	1.51 ± 0.02	0.13 ± 0.04	5.12 ± 0.03
	10	3.024 ± 0.04	1.29 ± 0.02	BDL	4.32 ± 0.02
	15	2.254 ± 0.05	0.96 ± 0.03	BDL	3.22 ± 0.03
	20	1.533 ± 0.02	0.65 ± 0.04	BDL	2.19 ± 0.03
	25	1.407 ± 0.02	0.60 ± 0.02	BDL	2.01 ± 0.02
	30	0.784 ± 0.01	0.33 ± 0.01	BDL	1.12 ± 0.01
FYM amendment					
	0	6.80 ± 0.03	2.92 ± 0.02	ND	9.72 ± 0.02
	3	5.53 ± 0.02	2.48 ± 0.03	0.27 ± 0.02	8.28 ± 0.02
	5	4.82 ± 0.03	2.01 ± 0.04	0.40 ± 0.03	7.23 ± 0.03
	7	3.48 ± 0.03	1.51 ± 0.03	0.13 ± 0.02	5.12 ± 0.02
	10	3.03 ± 0.04	1.29 ± 0.06	BDL	4.32 ± 0.04
	15	2.25 ± 0.05	0.97 ± 0.05	BDL	3.22 ± 0.05
	20	1.53 ± 0.05	0.66 ± 0.02	BDL	2.19 ± 0.03
	25	1.41 ± 0.02	0.60 ± 0.02	BDL	2.01 ± 0.02
	30	0.78 ± 0.07	0.34 ± 0.05	BDL	1.12 ± 0.04
Amendment charcoal					
	0	6.91 ± 0.01	2.96 ± 0.02	ND	9.87 ± 0.02
	3	6.22 ± 0.03	2.46 ± 0.02	0.21 ± 0.01	8.89 ± 0.02
	5	5.44 ± 0.05	2.32 ± 0.03	0.15 ± 0.01	7.91 ± 0.03
	7	4.49 ± 0.02	1.82 ± 0.05	0.10 ± 0.03	6.41 ± 0.03
	10	3.68 ± 0.04	1.58 ± 0.04	BDL	5.26 ± 0.04
	15	3.39 ± 0.01	1.46 ± 0.02	BDL	4.85 ± 0.02
	20	2.74 ± 0.02	1.17 ± 0.03	BDL	3.91 ± 0.02
	25	2.48 ± 0.03	1.06 ± 0.01	BDL	3.54 ± 0.02
	30	2.18 ± 0.03	0.94 ± 0.01	BDL	3.12 ± 0.02
Amendment Sea babul leaves					
	0	6.83 ± 0.03	2.93 ± 0.02	ND	9.76 ± 0.02
	3	5.80 ± 0.03	2.59 ± 0.04	0.25 ± 0.01	8.64 ± 0.02
	5	5.33 ± 0.02	2.19 ± 0.03	0.13 ± 0.01	7.65 ± 0.03
	7	4.62 ± 0.04	2.01 ± 0.04	0.08 ± 0.01	6.71 ± 0.03
	10	4.35 ± 0.02	1.86 ± 0.02	BDL	6.21 ± 0.04
	15	3.99 ± 0.05	1.71 ± 0.04	BDL	5.70 ± 0.02
	20	3.21 ± 0.03	1.37 ± 0.03	BDL	4.58 ± 0.02
	25	3.70 ± 0.02	1.16 ± 0.02	BDL	4.86 ± 0.03
	30	1.41 ± 0.03	0.60 ± 0.01	BDL	2.01 ± 0.02
Untreated control					
	0	6.88 ± 0.02	2.81 ± 0.01	ND	9.69 ± 0.02
	3	6.42 ± 0.03	2.39 ± 0.01	0.18 ± 0.02	8.99 ± 0.02
	5	5.55 ± 0.04	2.48 ± 0.02	0.13 ± 0.03	8.16 ± 0.03
	7	5.01 ± 0.05	2.34 ± 0.03	0.12 ± 0.03	7.47 ± 0.03
	10	4.22 ± 0.02	2.01 ± 0.04	BDL	6.23 ± 0.03
	15	3.44 ± 0.03	1.53 ± 0.04	BDL	4.97 ± 0.03

Table 2 continued

Amendment	Sampling days	Average residues (mg/kg)			
		α-endosulfan	β endosulfan	Endosulfan sulfate	Total
	20	2.84 ± 0.04	1.33 ± 0.03	BDL	4.17 ± 0.03
	25	2.58 ± 0.02	1.17 ± 0.02	BDL	3.75 ± 0.02
	30	2.38 ± 0.03	0.96 ± 0.02	BDL	3.34 ± 0.02

with vermin compost, FYM treated soil, charcoal amended soil and sea babul treated soil, respectively. The results of initial percent dissipation of soil amendments are presented in Table 2. The results indicated initial percent dissipation of endosulfan by day 5 was 25.62, 22.62, 19.86 and 21.62, in the soil amended with vermin compost, FYM, charcoal, leaves of sea babul tree, respectively, as compared to un-amended treatment where only 7.22 % dissipation of endosulfan was recorded (Table 2). The β endosulfan dissipated slowly as compared to α-endosulfan, as beta endosulfan possesses higher persistence in nature and soil. The metabolite of endosulfan, sulfate was detected on day -3 and dissipated by day -5 in each treatment. (Table 2). The metabolites of endosulfan, endosulfan ether, endosulfan lactone were not quantified, as they are non—toxic in nature. These treatments serve as bio-stimulators for the degradation process in soil. In 30-day incubation period the degradation was fastest in vermin compost, 88.48 %, followed by FYM, seababul leaves and charcoal, recording 87.21 %, 79.41 % and 68.39 %, respectively, as compared to un amended treatment where slow degradation of 65.53 % was observed (Fig. 2). Dissipation data for endosulfan under different amendments fitted well to first order kinetic equation, log $(C/C_0) = -K_{obs}t$, where C_0 is the initial concentration of the herbicide (lg/g), C is the concentration (lg/g) after time in days (t) (Table 2). The half life was 18.8 days in un-amended soil as compared to

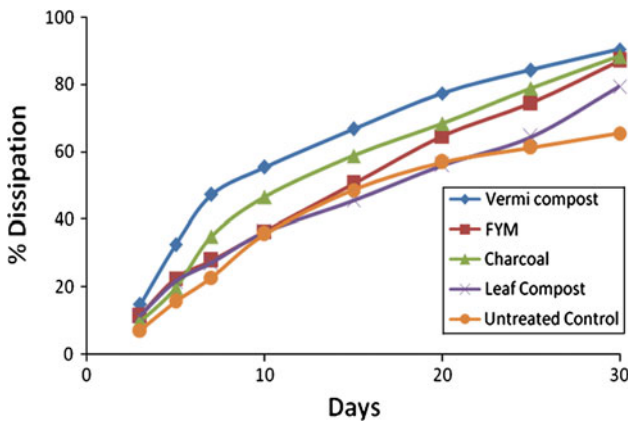

Fig. 2 Dissipation of endosulfan in soil under different organic amendments

Table 3 Regression equation and half life of endosulfan in soil in different amendments

Soil treatments	Regression equation Y=	Half life	Corelation coefficient
Untreated control soil	−0.016x + 0.980	18.81	0.98
Vermi compost	−0.2996x + 8.9013	1.01	0.97
Farm yard manure	−0.2422x + 9.1643	1.24	0.98
Leaf compost	−0.019x + 0.997	15.84	0.93
Charcoal	−0.2338x + 8.8693	1.29	0.92

1.01–1.29 days in vermi compost, farm yard manure, charcoal and leaf compost, (Fig. 2). The amendment with vermin compost, followed by FYM was found to be most effective and enhances the degradation as compared to the other amendments.

In light of the findings of this study, it could be concluded that the dissipation of endosulfan was favored by the alkaline soil conditions (pH 8), proper moisture and temperature levels that encouraged the microbial growth and degradation of both isomers of endosulfan. Since all treatments were subjected to the same levels of moisture and temperature, the variations in the rate of degradation between treatments were due to the differences in the organic amendments and in particular their effect on the microbial population and activity. It is expected that the dissipation of endosulfan in soil under field conditions will take a longer time. However, the addition of organic substances such as vermi compost and FYM will speed up the rate of decomposition for both isomers (Table 3).

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