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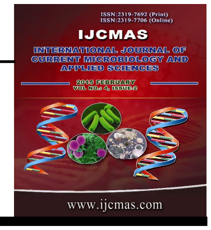

Original Research Article

Effect of Pesticides Alone and in Combinations on Sulphur Oxidation in Soils

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A B S T R A C T

Keywords

Sulphur oxidation, insecticides, fungicides, soils

A laboratory experiment was conducted to study the interaction effects of insecticides alone and in combination with fungicides on the sulphur oxidation in groundnut (*Arachis hypogaea* L.) soils collected from the Anantapur district of Andhra Pradesh, India. The rate of sulphur oxidation was dramatically increased at 2.5 kg ha^{-1}, whereas increase in the concentration of pesticides at 7.5 to 10 kg ha^{-1} level drastically decreased the rate of sulphur oxidation. The sulphur oxidation was pronounced more at 14-day of incubation with pesticides alone and in combination in both black and red soils. The oxidation of sulphur was significantly enhanced by the application monocrotophos in combination with mancozeb after 7 and 14-day of incubation in black soil.

Introduction

Sulphur is an essential component for agricultural crop production. In view of plant nutrition, sulphur stands next to nitrogen and phosphorus. Sulphur enters soil primarily in the form of plant residues and chemical fertilizers. A large part of the sulphur in the soil profile is present in organic matter. Sulphur transformation is mediated by chemolithotrophs and has become increasingly apparent in recent years, since sulphate is the plant available source of sulphur and demand for high sulphur content by commercial crops has been reported (Wen *et al.*, 2001). Despite the economic importance of sulphur, knowledge about pesticidal influence towards these microbial activities is scarce (Min *et al.*, 2001; Rohwerder *et al.*, 2003). Sulphur is an important component of the environment, and there is a usual cycle of oxidation and reduction reactions which transforms sulphur into organic and inorganic products. Elemental sulphur is gradually converted to sulphate in soil by the action of bacteria.

Sulphur is an essential element for all biological systems and has been recognized as a major nutrient for optimal plant growth. It is used by plant for synthesis of amino acid, biotin, thimate glutathione, co-enzyme–A, formation of chlorophyll, glucoside oils, disulphide and sulphuryl groups and activation of sulphurylase. Sulphur fertilization is a vital part of modern agriculture (Pradip *et al.,* 2011). It leads to increase the crude protein content of forages, oil content of oilseeds, persistence of legumes' stand and winter hardiness and drought tolerance of crop plants. It also improve quality of cereals, uniformity and quality of vegetables, control of some soil borne diseases and ultimately to higher yield.

Total sulphur may indicate the total pool of available sulphur in soil but it has got little value in describing short time availability of sulphur in soil. Therefore, the element, to get available to plant, is readily metabolized in soil in a cyclic manner (Pradip *et al.*, 2011). There are four distinct processes of organic compound decomposition; microbial assimilation or immobilization of simple compounds of sulphur; oxidation of inorganic compounds such as sulphide (S^{2-}), thiosulphate ($S_2O_2^-$), sulphite (SO_3^{2-}), polythionates and elemental sulphur; reduction of sulphate and other anions of sulphide. Apart from these abiotic factors, one of the major process to convert sulphur in available form for plant uptake is microbial oxidation, mainly by thiosulphate oxidizing bacteria, of the unavailable element and reduced sulphur to plant available SO_4^{2-} (Jensen *et al.,* 1995).

Materials and methods

Soils

Samples of black and red soils, collected from groundnut cultivated fields of Anantapur District in a semi-arid region of Andhra Pradesh, India from the depth of 12 cm, were air-dried and sieved through a 2 mm mesh screen before use.

Pesticides

In order to determine the influence of pesticides on sulphur oxidation, monocrotophos, chlorpyrifos alone and in combination with fungicides (monocrotophos + mancozeb and chlorpyrifos + carbendazim), were selected in the present study. For incubation studies and estimation of sulphate in soils, commercial formulations of the tested pesticides dissolved in distilled water were used. Chemical structures of the selected pesticides were represented in Fig.1.

Soil incubation

The soil ecosystem stimulating non-flooded conditions consisting of ten gram portions of soil samples were added in test tubes (25 x 150 mm) and soil samples were moistened to maintain at 60% water holding capacity. Same model was used previously, to elucidate the effects of insecticides on microbial activities by Tu *et al.* (1996); Rangaswamy and Venkateswarlu (2000); Raymond *et al.* (2003) and Jaya Madhuri and Rangaswamy (2003).

Statistical analysis

All data are averages of three replicates. The data were analyzed for significant differences ($P \leq 0.05$) between pesticides treated and untreated soil samples using Duncan's multiple range (DMR) test (Srinivasulu *et al*., 2012).

Estimation of sulphate

Ten gram portions of soil samples were suspended in 100 ml sodium acetate - acetic acid buffer solution, thoroughly agitated in a wrist action shaker for 30 minutes, and the soil suspensions were passed through Whatman filter paper No. 42. Suitable aliquot of the filtrate was pipetted into 25 ml volumetric flask. Then 2.5 ml of 25 HNO_3 and 20 ml acetic phosphoric acid (3 parts of AR grade acetic acid + 1 part of AR grade H_3PO_4) mixture was added to each flask and diluted to about 22 ml. After shaking the contents thoroughly, 0.5 ml of $BaSO_4$ seed suspension (18 g AR grade $BaCl_2$ was dissolved in 44 ml of hot distilled water and 0.5 ml of standard sulphate solution (2 mg /ml) were added and the solution was then boiled, cooled and 4 ml of gum acacia - acetic acid solution was added. Then 0.2 g AR grade $BaCl_2$ crystals (passed through a 1 mm sieve) were added and mixed thoroughly. After 10 minutes, 1 ml of gum acacia - acetic acid solution was added (5 g of gum acacia was dissolved in 500 ml hot distilled water, filtered through Whatman No. 42 filter paper and the filtrate was cooled and its volume was made to one litre with AR grade acetic acid) and set aside for 90 minutes. Then the flasks were inverted ten times and the absorbance was read in a Spectronic 20-D spectrophotometer at 440 nm.

Result and Discussion

Soil application of pesticides, singly and in combinations, significantly enhanced the oxidation of sulphur in terms of sulphate formed when applied at 2.5 kg ha^{-1}, whereas increase in the concentration of pesticides up to 10.0 kg ha^{-1} resulted in a decrease in the sulphur oxidation was observed in black and red soils (Table 1 and 2). Significant stimulation in sulphur oxidation occurred after incubation for 14 days in both soils. Treatments receiving monocrotophos, chlorpyrifos, monocrotophos + mancozeb, and chlorpyrifos + carbendazim, showed a highest sulphur oxidation rate at 2.5 kg ha^{-1} relative to the control after incubation for 14 days in both black and red soils (Table 1 and 2). Overall the rate of sulphur oxidation was increased when applied the insecticides alone and in combination with fungicides.

The significant enhancement in the formation sulphate from sulphur was observed in black soil than in red soil due to the presence of highest organic matter content in the black soil. The oxidation sulphur was initially low and then gradually increased up to 2.5 kg ha^{-1} of insecticides (monocrotophos, chlorpyrifos) alone and in combination with fungicides (monocrotophos + mancozeb, Chlorpyrifos + carbendazim) and decreased gradually by the increase in the concentration of pesticides up to 7.5 and 10 kg ha^{-1}. The oxidation of sulphur was increased in case of all individual and binary mixtures of pesticides significantly after 14 days of incubation in comparison to 7-day incubated soils. The results of the study indicate that, application of insecticides alone and combination with fungicides profoundly enhanced the rate of sulphur oxidation when applied the pesticides at recommended levels.

Microbial transformation of sulphur regulate the bioavailability, toxicity and environmental impact of these elements in the biosphere. Sulphur oxidation provides microorganisms with nutrient or energy source (Germida and Siciliano, 2003). Sulphur oxidation in soil is restricted by a number of factors like aerobic, anaerobic conditions, presence of chloride ions, carbon dioxide level etc. Large scale applications of the pesticides in agricultural soils seldom effect the sulphur oxidizers, in particular species of *thiobacilli*. Any such treatment which alters the number of sulphur oxidizing bacteria, is likely to influence the rate of sulphur oxidation and the amount of plant available sulphate-sulphur. Despite the major role played by sulphur in soil biogeochemical cycles, only few isolated studies were made on the effect of pesticides towards microbial activity implicated in sulphur oxidation. Initially, Sivasithamparam (1969, 1970) found that chlorpyrifos treated soil showed a distinct increase in the number of sulphate oxidizers thereby stimulating microbial oxidation of sulphur, predominantly after three months. Same phenomenon was observed with the above pesticide at 1 and 250 µg g^{-1} soil (Wainwright, 1979). In the same manner, addition of the commercial formulation of benomyl in alluvial and laterite soils under flooded conditions enhanced the sulphur oxidizing activity at 5 and 10 µg g^{-1} soil (Ray *et al*., 1980). Tu (1994) and Tu *et al.* (1996) have also demonstrated that the fungicides dodine, chlorothalonil, folpet, thiram and captan increased sulphur oxidation in loamy sand soils under lab conditions. Likewise, the population of sulphur oxidizing strains increased in the presence of malathion and dimethoate (Bezbaruah *et al*., 1995). This kind of stimulation was further reported by Tu (1996) on treatment with herbicides during 8-week period in the sandy loam soil. Furthermore, experimental results of Rangaswamy and Venkateswarlu (1999) indicated that monocrotophos, quinalphos, cypermethrin and fenvalerate promoted the microbial activity in groundnut cultivated soils. Besides these, Min *et al*., (2001) reported that the influence of the herbicide butachlor (22 µg/g dried soil) is a positive effect on sulphur oxidizing bacteria. Contradictory to the stimulative nature of pesticides, Tu (1970; 1972; 1973a, b) stated that carbofuran, chlorpyrifos, diazinon, ethoprop, fensulfothion, thionazin and trichloronate had little or no significant deleterious effect on sulphur oxidation in a sandy loam soil.

In contrast, to the progressive increase or non-toxic effect in sulphur oxidizing activity after pesticide application, reports of inhibition at high concentration was supported by Tu and Bollen (1968). Tu (1968) noticed that aldrin and dieldrin at 2000 ppm reduced the rate of sulphur. Tu and Bollen (1968) observed that paraquat inhibited sulphur oxidation when applied at high rates. Tu (1970) also reported that zinophos and dursban depressed sulphur oxidizing activity 12 and 17% respectively, at a higher concentration of 100 µg/g soil. In similarity to this, carbofuran at 250 µg/g caused initial inhibition followed by increase in sulphate formation (Wainwright, 1979). Ray and Sethunathan (1989) stated that HCH (5 ppm) and benomyl (10 ppm) inhibited the oxidation of elemental sulphur in unsaturated soil amended with elemental sulphur. Ray (1991) denoted that hinosan at 25 ppm reduced sulphur oxidation which did not affect after 20 days. Further, Tu (1992) demonstrated that the pesticides haloxyfrop and pyroxyfur at 10 fg/g of soil applied to a sandy loam caused reduction in the level of sulphur oxidation. Similar type of reduced activity was suggested by Bezbaruah *et al.* (1995) with fenitrothion and endosulfan.

The results obtained in the present investigation clearly indicate that the application of insecticides alone and combination with fungicides significantly enhanced the rate of sulphur oxidation specifically at 2.5 kg ha^{-1} in both black and red soils. The effective combination for the stimulation of sulphur oxidation was monocrotophos+mancozeb in black soil. It is concluded that the application insecticides alone (monocrotophos, chlorpyrifos) and in combination with fungicides (monocrotophos+mancozeb, chlorpyrifos +carbendazim) at field application rates (2.5 to 5.0 kg ha^{-1}) improves the sulphur oxidation in black and red soil.

Table.1 Influence of insecticides alone and in combination with fungicides on sulphur oxidation in black soil

Pesticide	Soil incubation in days, after pesticide application											
	7 days						14 days					
	0*	1.0	2.5	5.0	7.5	10	0	1.0	2.5	5.0	7.5	10.0
Monocrotophos	605a	630b	706c	610d	550e	460	710a	735b	800c	760d	636e	508d
	(100)	(104)	(116)	(101)	(90)	(76)	(100)	(103)	(114)	(107)	(89)	(71)
Monocrotophos	605a	640b	680c	612d	580e	422	710a	730 b	805c	745d	608e	540f
+Mancozeb	(100)	(105)	(112)	(101)	(96)	(70)	(100)	(103)	(113)	(104)	(85)	(76)
Chlorpyrifos	605a	626b	701c	685c	560d	500e	710a	740b	820c	754d	615e	520f
	(100)	(103)	(115)	(112)	(92)	(82)	(100)	(104)	(115)	(106)	(86)	(73)
Chlopyrifos	605a	620b	660c	530d	500e	435f	710a	752b	815c	748d	622e	535f
+Carbendazim	(100)	(102)	(109)	(875)	(82)	(78)	(100)	(106)	(114)	(105)	(87)	(75)

* Values µg SO_4^{2-} S g^{-1} soil formed after added sulphur of 2.5 mg g^{-1}

Figures, in parenthesis, indicate relative production percentages.

Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Table.2 Influence of insecticides alone and in combination with fungicides on sulphur oxidation in red soil

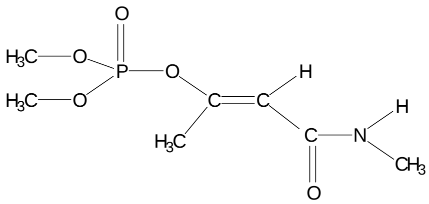
Pesticide	Soil incubation in days, after pesticide application											
	7 days						14 days					
	0*	1.0	2.5	5.0	7.5	10	0	1.0	2.5	5.0	7.5	10.0
Monocrotophos	450a	508b	612c	580d	500b	410e	502	580	690c	510d	452e	400f
	(100)	(113)	(135)	(128)	(111)	(91)	(100)	(115)	(137)	(101)	(90)	(78)
Monocrotophos	450a	460b	503c	420d	400d	356e	502	530b	640c	491d	430e	390f
+Mancozeb	(100)	(102)	(112)	(93)	(89)	(79)	(100)	(105)	(127)	(98)	(85)	(78)
Chlorpyrifos	450a	455b	491c	430d	381d	312e	502	510b	530c	480d	410e	372f
	(100)	(101)	109()	(95)	(85)	(69)	(100)	(101)	(105)	(96)	(82)	(74)
Chlopyrifos	450a	455	470	410d	390a	352e	502	508b	520c	473d	400e	352f
+Carbendazim	(100)	(101)	(104)	(91)	(87)	(78)	(100)	(101)	(103)	(94)	(78)	(70)

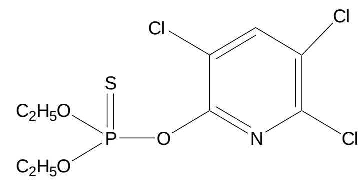
* Values µg SO_4^{2-} S g^{-1} soil formed after added sulphur of 2.5 mg g^{-1}

Figures, in parenthesis, indicate relative production percentages.

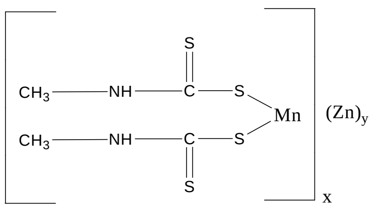
Means, in each row, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

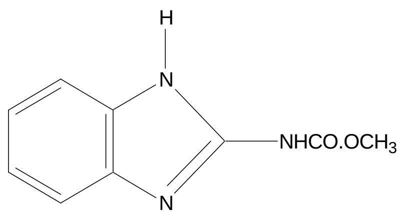
Fig.1 Chemical structures of pesticides used in the present study





Monocrotophos Chlorpyrifos





Mancozeb Carbendazim

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