Indian Journal of Agricultural Sciences **80** (7): 640–4, July 2010

Effect of pesticides on biochemical properties of tomato (*Solanum lycopersicon*) cropped soil

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Received: 31 March 2009; Accepted: 29 April 2010

ABSTRACT

A study was conducted during 2006–08 on the effect of pesticides (mancozeb, endosulfan and chlorpyrifos) on tomato (*Solanum lycopersicon* L.) cropped soil for total microbial population, symbiotic and asymbiotic nitrogen bacterial counts and microbial activities. The physico-chemical soil properties were not affected by pesticide applications, however, the viable microbial counts were reduced drastically even at lower concentrations of applied pesticides. All pesticides had moderate to high inhibitory effect on phosphatase and dehydrogenase activity, though the effect of chlorpyrifos was most persistent. Chlorpyrifos at 100 mg/kg inhibited more than 35 and 50% activity of dehydrogenase and phosphatase enzymes, respectively, even after 35 days of treatment. Mancozeb was highly toxic at the initial stage, inhibiting 90% dehydrogenase and 65% phosphatase activity within 7 days of treatment at 100 mg/kg. The microbial respiratory activity was also highly affected in the presence of all the pesticides at 50 and 100 mg/kg levels. After 1 day of pesticide treatment, CO_2 evaluation was minimum (1 mg CO_2/50 g soil) in mancozeb.

Key words: Dehydrogenase, Microbial population, Pesticides, Phosphatase, Soil properties

Pesticides are widely used against a range of pests infesting agricultural crops. Globally about 3×10^9 kg of pesticides is applied annually with a purchase price of nearly $40 billion (Pan 2003). The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem and affect human health by entering in the food chain (Little field Wyer *et al*. 2008). These pesticides also influence soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated bio-transformations, such as nutrient dynamics and their bio-availability are also more or less adversely affected by their use (Mahia *et al*. 2008, Niewiadomska 2004).

Soil microbial respiration and their enzymatic activities act as biological index of soil fertility. Soil respiration is measured in terms of CO_2 evolution, whereas phosphatase (both acid and alkaline phosphatase) and dehydrogenase assay has been recommended as an index of general activity of soil microorganisms and can be used as a sensitive marker of soil degradation. Most of the earlier reports indicated that pesticides frequently show inhibitory effects of microbial respiration and enzymes activity in soil (Maarit *et al*. 2009, Xie *et al*. 2004).

Mancozeb, endosulfan and chlorpyrifos are the recommended pesticides against tomato (*Solanum lycopersicon* L.) insect-pests and diseases in Himachal Pradesh (Anonymous 2009), however, there is no report indicating their effects on biological activities in soil. Therefore, the present investigation was undertaken to study the effect of these pesticides on microbial population, CO_2 evolution (microbial activity), and dehydrogenase and phosphatase activities in tomato cropped soil under *in vitro* conditions.

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MATERIALS AND METHODS

The fresh soil samples (2 kg each) were collected from tomato field (0–15 cm depth excluding any plant cover) in polyethylene bags. The soil was dried under shade, ground and sieved through a 2 mm mesh sieve to remove earthworms, arthropods, stones, plant roots and other debris. The physico-chemical properties, viz *p*H, EC (electrical conductivity) organic matter were determined as per standard methods (Jackson 1973). The preliminary analysis of the soil indicated 3.33% organic matter, 0.48 d S/m EC and 6.82 *p*H.

For pesticide studies, each soil sample (500 g) was mixed separately with 5, 50 and 100 mg/kg concentrations of different pesticides (mancozeb, endosulfan and chlorpyrifos). Every concentration was replicated 6 times. The known quantity of soil was adjusted to 50% of maximum water-holding capacity with deionized water and incubated at 28± 2°C for 35 days. Control soil was treated with deionized water only.

The enzymes (dehydrogenase and alkaline phosphatase) activity was studied at 7, 14, 21, 28 and 35 days after establishment of the experiment. The dehydrogenase activity was measured colorimetrically by using triphenyl tetrazolium chloride. The enzyme activity was expressed in terms of formazan produced and measured at 485 nm in Shimadzu UV/VIS spectrophotometer. The quantification of formazan was done from a standard curve by using triphenyl formazan (TPF, Sigma) in methanol. The alkaline phosphatase activity was also measured colorimetrically by using *p*-nitrophenyl phosphate as the substrate at 420 nm.

The soil respiration was measured by estimating CO_2 evolution as described by an indirect method to measure the microbial activity (Kukreja *et al*. 1991). The pesticide treated, sub-samples of 50 g soil were taken in a 1000 ml flask to assess effects of pesticides on the CO_2 evolution/oxygen consumption by the microorganisms. A tube containing 10 ml of 0.5 N NaOH was inserted into a flask containing 50 g soil. The flask was kept air tight and placed in an incubator at 28±2°C for a period of 35 days or till the recovery of microbes was reattained after the disruption of the treatment.

The total microbial population was studied on day 7 after the pesticide treatment by using serial dilution and plate method on specific media, viz fungi (Martin's Rose Bengal medium), actinomycetes (Kenknight and Munaier's), bacteria (Nutrient Agar), *Rhizobium* (Yeast Extract Manitol Agar (YEMA) and *Azotobacter* (Jensen's medium). The CRYEMA (Congo Red Yeast Extract Manitol Agar), BTB (Bromothymol Blue), Hofer's alkaline (*p*H 11.0) and plant infection test was also performed to have the authenticity of the Rhizobia from its common contaminants on YEMA medium. For identification of *Azotobacter*, presumptive tests were carried out following standard methods as outlined in Bergey's manual of Systematic Bacteriology (Krieg and Halt 1984).

Table 1 The *p*H and electrical conductivity of pesticide-treated soil

Treatment	Dose (mg/ kg)	*p*H	Electrical conductivity
Mancozeb	5	6.87 ± 0.18	0.45 ± 0.05
	50	6.71 ± 0.33	0.48 ± 0.13
	100	6.89 ± 0.03	0.59 ± 0.16
Endosulfan	5	7.04 ± 0.33	0.47 ± 0.08
	50	7.13 ± 0.20	0.51 ± 0.18
	100	7.20 ± 0.13	0.54 ± 0.20
Chlorpyrifos	5	6.78 ± 0.46	0.50 ± 0.17
	50	7.08 ± 0.03	0.51 ± 0.05
	100	7.22 ± 0.15	0.60 ± 0.09
Control	000	6.82 ± 0.15	0.48 ± 0.10

RESULTS AND DISCUSSION

Soils were neutral in reaction and having electric conductivity in the safer limit indicating no significant effect of pesticides on soil *p*H or electrical conductivity at applied concentrations (Table 1). These observations are in agreement with the findings of Gopal *et al*. (2001).

The effect of the pesticides on soil microbiological and biological properties was the main issue of research because of their impact on public health and soil quality. The data presented in Table 2 indicated that each pesticide had detrimental effect on microbial activity in soil. The dehydrogenase activity was minimum (0.7 µg TPF/g soil) in mancozeb (100 mg/kg) treatment, followed by 1 µg TPF/g soil in chlorpyrifos (100 mg/kg) treated soil after 7 days of treatment. Chlorpyrifos was the most persistent and its toxicity was found significantly higher (5.4 µg TPF/g soil) than other pesticides, irrespective of doses and duration.

Table 2 Effect of pesticides on soil dehydrogenase activity in tomato cropped soil

Treatment	Dose (mg/ kg)	µg of triphenyl formazan (TPF) released/g soil at indicated intervals (days)					
		7	14	21	28	35	Mean
Mancozeb	5	4.7	5.5	5.9	6.1	6.3	5.7^{ef}
	50	2.5	3.2	4.4	4.7	5.8	4.1^{d}
	100	0.7	2.0	2.7	4.3	5.3	3.0^{b}
Endosulfan	5	5.2	5.8	6.0	6.2	6.4	5.9^{f}
	50	3.6	3.7	4.1	4.5	5.5	4.3^{d}
	100	1.8	2.3	2.6	3.7	4.8	3.0^{b}
Chlorpyriphos	5	4.7	5.3	5.7	6.0	6.3	5.6^{e}
	50	2.7	3.1	3.6	4.1	4.8	3.7^{c}
	100	1.0	1.6	2.0	3.0	4.0	2.3^{a}
Control	000	5.9	6.2	6.3	6.5	6.5	6.3^{g}
Mean		3.3^{a}	3.9^{a}	4.3^{b}	4.9^{c}	5.6^{d}	
CD (*P*=0.05)	Dose (D) =		0.21				
	Interval (I) =		0.15				
	Dose × Interval (D × I) =		0.47				

Mean values superscripted with same alphabet do not differ significantly

Table 3 Effect of pesticides on soil phosphatase activity in the tomato cropped soil

Treatment	Dose (mg/ kg)	µg of p-nitrophenol released/g soil in indicated interval (days)					
		7	14	21	28	35	Mean
Mancozeb	5	8.8	9.5	9.6	10.7	11.2	9.9^{e}
	50	5.3	7.5	7.9	10.1	10.5	8.3^{c}
	100	3.5	5.4	6.7	9.0	9.6	6.8^{b}
Endosulfan	5	9.1	9.6	9.8	10.3	7.9	9.3^{de}
	50	6.9	7.7	8.5	9.6	11.1	8.7^{cd}
	100	5.6	6.1	7.4	8.3	10.3	7.5^{b}
Chlorpyrifos	5	9.0	9.3	9.9	10.2	10.5	9.8^{e}
	50	5.7	6.5	7.5	8.4	9.4	7.5^{b}
	100	3.9	4.3	5.2	6.2	7.7	5.4^{a}
Control	000	10.4	10.9	11.0	11.4	11.7	11.1^{f}
Mean		6.8^{a}	7.7^{b}	8.4^{c}	9.4^{d}	9.9^{d}	
CD (*P*=0.05)	Dose (D) =	0.71					
	Interval (I) =	0.50					
	Dose × interval (D × I) =	1.58					

Mean values superscripted with same alphabet do not differ significantly

Endosulfan reduced the TPF level to 1.8 and 3.6 µg at 100 and 50 mg/kg levels, respectively, after 7 days of treatment. Up to 14 days of treatment, at 50 and 100 mg/kg levels, all pesticides were highly toxic, thereafter, the extent of toxicity gradually decreased with the increase in incubation period.

Similarly, application of different concentrations of each pesticide significantly reduced phosphatase activity (Table 3). Initially mancozeb was found to be highly toxic (3.5 µg p – nitro phenol/g soil, after 7 days) at 100 mg/kg level. However, with the increase of incubation period beyond 14 days, its toxicity decreased at the same concentration. A similar trend was observed in endosulfan and chlorpyrifos. Chlorpyrifos, irrespective of incubation intervals, significantly reduced the phosphatase activity to a lower level (5.4 µg *p*-nitro phenol/g soil) and was the most toxic as compared to other treatments. At 5 mg/kg level, the toxicity of each pesticide to phosphatase enzyme (9.3–9.9 µg p-nitro phenol) was at par but significantly different from control.

The present findings indicated a significant difference not only among the different doses of each pesticide but also between pesticides with regards to their ecological effects on soil biological activities. Regarding exposure time, significant difference was found to exist among 7, 14, 21, 28 and 35 days for dehydrogenase, phosphotase and respiratory activities. The study indicated that the effect of pesticides was more prominent on soil micro flora during 7 to 14 days and thereafter, decreased gradually towards the end of the experimentation (35 days).

The studies conducted envisaged that each pesticide behaved differently in their toxic effect on microbial respiratory activity of treated soil (Fig 1). After one day of mancozeb treatment (100 mg/kg level), CO_2 evolution receded to its maximum (1 mg/50 g soil), whereas, it was 3 mg/50 g soil on the same day in chlorpyrifos at the same concentration. The lowest concentration (5 mg/kg) of these pesticides was least toxic as it reduced the CO_2 emission to

Table 4 Effect of pesticides on the microbial population in tomato cropped soil

Treatment	Dose (mg/kg)	Microbial counts/g soil after 7 days of treatment				
		Bacteria	Fungi	Actinomycetes	*Rhizobium* sp.	*Azotobacter* sp.
Mancozeb	5	2.57×10^4(97.73)	0.19×10^4(98.55)	0.67×10^4(96.16)	3.84×10^4(98.82)	0.66×10^4(97.97)
	50	1.71×10^4(98.49)	0.04×10^4(99.69)	ND (100.00)	3.56×10^4(98.90)	0.33×10^4(98.98)
	100	0.34×10^4(99.70)	0.01×10^4(99.92)	ND (100.00)	3.00×10^4(99.08)	0.17×10^4(99.48)
Endosulfan	5	3.00×10^4(97.35)	3.10×10^4 (76.30)	0.90×10^4(89.47)	3.76×10^4(98.84)	0.76×10^4(97.66)
	50	2.69×10^4(97.63)	0.30×10^4(97.71)	0.62×10^4(92.75)	3.58×10^4(98.90)	0.37×10^4(98.86)
	100	0.64×10^4(99.44)	0.17×10^4(98.70)	0.35×10^4(95.91)	3.00×10^4(99.08	0.20×10^4(99.38)
Chlorpyrifos	5	2.12×10^4(98.13)	0.33×10^4(97.48)	0.21×10^4(97.54)	3.64×10^4(98.88)	0.56×10^4(98.28)
	50	0.69×10^4(99.39)	0.15×10^4(98.85)	ND(100.00)	3.45×10^4(98.94)	0.30×10^4(99.07)
	100	0.15×10^4(99.87)	0.11×10^4(99.16)	ND(100.00)	3.00×10^4(99.08)	0.14×10^4(99.57)
Control	000	113.30×10^4	13.08×10^4	8.55×10^4	325×10^4	32.5×10^4

Figures in parentheses are per cent decrease in microbial counts over control

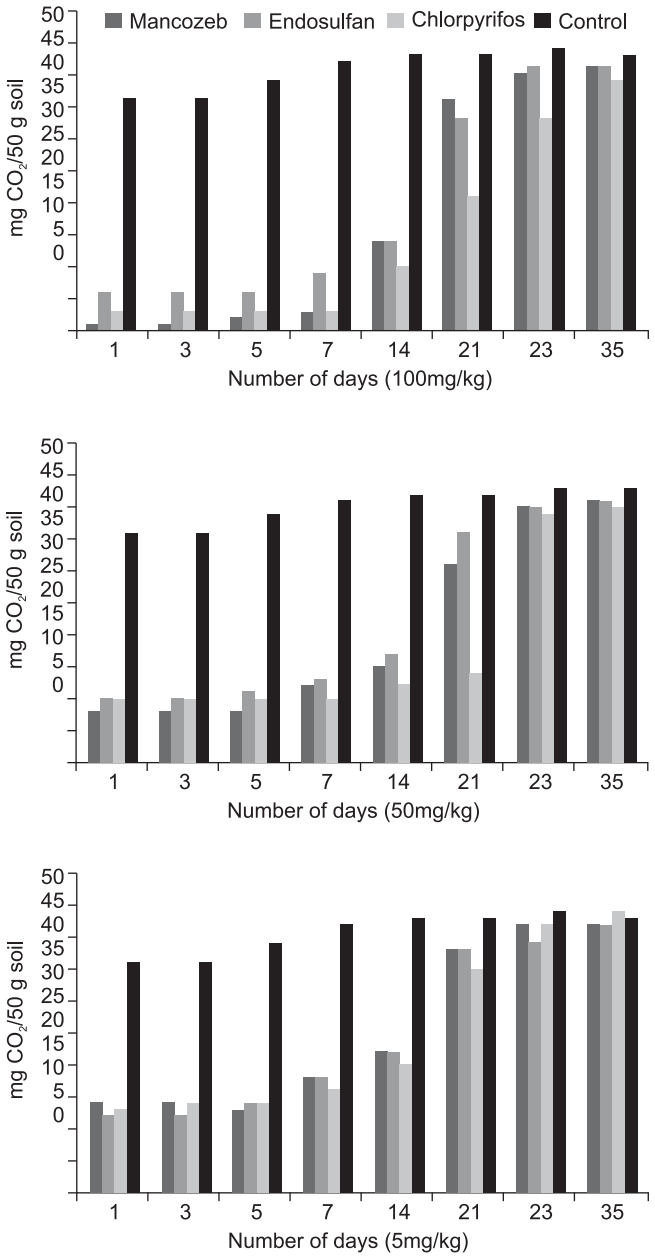

Fig 1 Effect of pesticides on microbial respiration in tomato cropped soil

15–17 mg/50 g soil compared to control (41 mg/50 g soil), after 7 days of treatment. However, higher concentrations of these pesticides (50 and 100 mg/kg) were highly toxic and decreased the CO_2 emission/oxygen uptake significantly at different stages of observations till 14 days. Afterwards, this emission/uptake increased gradually and reached to almost equilibrium state till 35 days of treatment (Fig 1).

The soil micro flora (bacteria, fungi, actinomycetes, symbiotic (*Rhizobium*) and non-symbiotic (*Azotobacter* spp) (N-fixers) were very susceptible/sensitive to all the applied pesticides as shown by the extreme toxicity (Table 4). The per cent mortality for these soil microbes ranged from 97 to 100, irrespective of doses and pesticides. The mortality increased with increase in pesticide concentration. However, the microbial activity, in general, seemed to recover partially after 2 weeks following application of pesticides. Kalam and Mukherjee (2001, 2002) also reported reduction in viable counts of bacteria (symbiotic and asymbiotic free living N-fixers), fungi and actinomycetes by the pesticides application. Chlorpyrifos has also been found to reduce the population of bacteria, fungi and actinomycetes significantly over control by Chu *et al*. (2008).

The pesticides interact with soil organisms and their enzymatic/metabolic activities alter the physiological, biochemical and ecological processes (Schultz and Urban 2008, Singh and Walker 2006). Phosphatase activity of growth-promoting fungi get inhibited in the presence of insecticides, while promoted in the presence of fungicides like afugam and tilt (Omar and Abd-Alla 2000). Higher concentration of endosulfan and methyl parathion cause inhibition of enzyme activities like amylase, protease, phosphatase etc. (Sabale and Misal 2000). However, the present investigations revealed that mancozeb, endosulfan and chlorpyrifos not only inhibited respiratory activity (about 60%), phosphatase and dehydrogenase enzymes (about 11–20%), but also proved detrimental to soil bacteria, fungi, actinomycetes, *Rhizobium* and *Azotobacter* spp even at 5 mg/kg. The results confirm the findings of Kalam and Mukherjee (2001 and 2002). Adverse impacts of pesticides on soil microbial diversity and activities have also been reported by Singh and Walker (2006) and Littlefield *et al*. (2008).

Different concentrations/doses of pesticides, viz mancozeb, endosulfan and chlorpyrifos did not affect soil pH and electric conductivity (EC), however, they drastically reduced (97–100%) its flora as compared to untreated soil. The enzymes activity like dehydrogenase and phosphatase was also inhibited significantly in a dose dependent manner. The reduction was more pronounced up to 14 days of treatment and thereafter, the microbial activity started recovering. The inhibition was about 90% and 65% for respective enzymes within 7 days of treatment at 100 mg/kg. The long-term application of such pesticides can disturb the biochemical equilibrium vis-à-vis soil fertility and productivity. Understanding the molecular responses of the microbes to toxicity of pesticide could be helpful in determining the risk assessment of pesticide contaminations and consequent adverse impacts on soil microbial diversity, enzymatic activity and biochemical reactions. Such pesticide-soil microbial interaction studies need to be carried out on long-term basis to identify toxic pesticides for their future review.

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