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EFFECT OF INSECTICIDES ON SOIL MICROORGANISMS

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ABSTRACT

Masum Billah AA, Ferdous MH, Taufique T, Mehraj H, Jamal Uddin AFM (2014) Effect of insecticides on soil microorganisms. *Int. J. Expt. Agric.* 4(4), 24-28.

An experiment was conducted in the laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Bangladesh from July to December, 2008 to know the impact of 4 selected insecticides (i.e., Diazinon 60 EC, Marshal 20 EC, Dursban 20 EC and Admire 200 SL) on soil microorganisms. The impact was expressed as amount of CO_2 evolution/gram soil and dehydrogenase activity (TPF formation). CO_2 evolution in soils indicates a great activity of soil microorganisms and maximum CO_2 evolution was found from Marshal 20 EC (74.8, 281.2 and 142.4 µg/g soil) at 2, 8 and 24 days of incubation while Admire 200 SL (126.5 and 222.4 µg/g soil) at 4 and 16 days of incubation. Diazinon 60 EC (0.073, 0.075, 0.079, 0.072 and 0.067 µmole/ml at 2, 4, 8, 16 and 24 days of incubation respectively) had maximum TPF formation.

Key words: *CO_2 evolution, TPF formation and incubation*

INTRODUCTION

Microorganisms make up appreciably less than 1% of total soil volume (Purohit 2003) and they can be categorized into bacteria, actinomycetes, fungi, algae and protozoa (Rao 1995). Soil respiration is a good index of the activity of microorganisms involved in organic matter decomposition (Komal *et al.* 1999). Soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulphur and phosphorus cycles (Klein *et al.* 1971). Synthetic insecticides are generally organic compounds and microorganisms are able to break down these compounds by their enzymes and utilize carbon as a source of energy (Purohit 2003). Dehydrogenases are essential components of the enzyme systems of microorganisms and their activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in soil (Stevenson 1956). The continuous and repeated use of insecticides affects the microbial population, respiration, soil dehydrogenase activity, nitrogen fixation, ammonification, nitrification and enzymatic activities in soil (Bujin and Yongxi, 2000). This consequently affects the soil health, fertility, productivity and crop yield. Some insecticides favored (Das *et al.* 1995; Bujin and Yongxi, 2000; Digrak and Kazanici, 2001; Das and Mukherjee, 2000; Latif 2007) and some exerted adverse effects (Ahtiainen *et al.* 2003; Digrak and Kazanici, 2001; Latif 2007; Tu 1980; Bhuyan *et al.* 1992; Martinez-Toledo *et al.* 1995; Komal *et al.* 1999) on the growth and activities of microorganisms in soil. As the insecticides applied in the soil, changes in the soil microbial activity and soil microbial populations (Rao 1995) affect the soil dehydrogenase activity. Therefore, the present study was under taken to know the effect of some selected insecticides on soil microorganisms.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, during the period from July to December, 2008. Soil sample was collected from the farm of SAU and brought to the laboratory. Then spread for partial air drying after removal of plant roots, insects, worms and small pieces of organic matter and passed through 2 mm mesh sieve. After sieving, soil was pre-incubated in the laboratory.

Pre-incubation of soil: Soil with 40% water holding capacity was subjected to pre-incubation aerobically at room temperature for 10 days. This allowed the soil microbial population to stabilize, minimize the effects of soil handling and preparation (Chowdhury *et al*. 1999). Immediately after conditioning the soil was used for the experiment.

Four treatments *viz*., Diazinon 60 EC @ 3.0 ml/L, Marshal 20 EC @ 2.0 ml/L, Dursban 20 EC @ 2.0 ml/L, Admire 200 SL @ 4.0 ml/L and control (untreated) were used in the experiment following a Completely Randomized Design (CRD) with three replications.

Procedure for determination of soil microbial activity: Sixty gram dry soil was weighed in a 100 ml glass jar and 5 ml of insecticide solution was injected inside the soil of the jar for each treatment. Five milliliters of distilled water was added to the control soil (untreated) to maintain moisture content equivalent to those of treated soils. Following insecticide application, glass jars were placed in 1L glass bottles. To trap CO_2 evolved by soil microorganisms during each incubation, 20 ml of 1M NaOH solution was taken in a small glass bottle and placed inside the jar. The 1L glass bottles were sealed and incubated for 24 days at room temperature. To maintain internal humidity of 1L glass bottle, 10 ml of distilled water was added at the bottom of each

incubation bottle. The amount of CO_2 evolved due to soil microbial respiration was determined after 2, 4, 8, 16 and 24 days of incubation.

Microbial respiration: Microbial respiration was monitored as CO_2 evolution from soil samples after 2, 4, 8, 16 and 24 days of incubation. Fresh NaOH was replaced at each sampling. The trapped CO_2 was titrated with standard (0.09N) HC1 using pH meter. Microbial activity was expressed as µg CO_2-C evolved g^{-1} soil. Following reactions occurred during titration with HCl.

1.	$NaOH + CO_2$	→	$Na_2CO_3 + NaHCO_3 + H_2O$	pH = 12
2.	$Na_2CO_3 + HCl$	→	$NaHCO_3 + NaCl$	pH = 12-8.3
3.	$NaHCO_3 + HCl$	→	$H_2CO_3 + NaCl$	pH = 8.3-3.7

The amount of total CO_2-C was determined by using the following formula:

Total CO_2-C = (X-B) × N ×12 × 20/Y × 1000/w, µg C/g soil

Where; X = Actual titration, B = Blank titration, N = Normality of HC1 (0.09236), 12 = Atomic weight of Carbon, 20 = Volume of NaOH, Y = Actual amount used for titration, 1000 = fig conversion and w = weight of soil in g (60 g soil).

Substrate solution (TTC), 1% TTC solution: 3 g of 2, 3, 5-triphenyltetrazolium chloride was dissolved in 100 ml ethanol. The solution was stored for one week at 4ºC in dark or in amber bottle.

Glucose solution: For preparing glucose solution 1 g of glucose was dissolved in 100 ml distilled water.

Triphenyl formazan (TPF) solutions: A standard solution of 0.0002 M triphenyl formazan (C6H5N:NC[C6H5]:NNHC6H5) was prepared by dissolving 0.03 g triphenyl formazan in 500 ml ethyl alchol. 1 g air dried representative soil sample was taken in an air tight screw capped test tube (15 ml capacity). 0.2 ml of 3% TTC solution was added to each of the tubes to saturate the soil. 0.5 ml of 1% glucose solution was added in each tube; gently tapped the bottom of the tube to drive out all trapped oxygen so that a water seal is formed above the soil. Ensure that no air bubbles are formed. The tubes were incubated at 28±0.5°C for 24 hours. After incubation, 10 ml of methanol was added, shaken vigorously and with stand for 6 hours. The clear pink coloured supernatant fluid was removed and the reading was taken with a spectrometer at wave length of 485 ηm. The amount of TPF formed was calculated from the standard curve (Fig. 1).

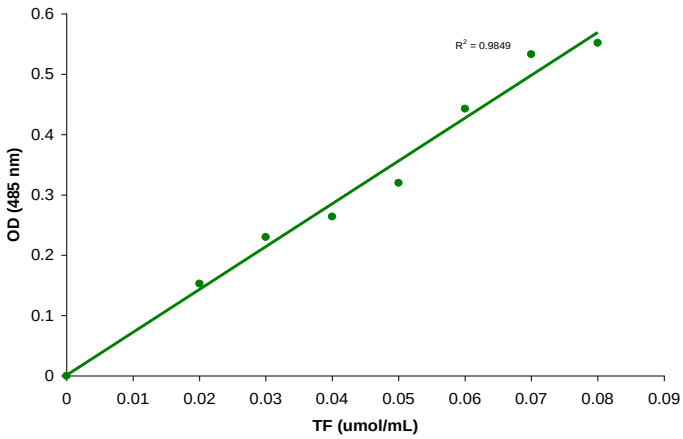

Fig. 1. Standard curve used for determination of the dehydrogenase activity in the tested soils

Collected data were statistically analyzed using MSTAT-C Computer Package Program. The significance of the differences among the treatment means were estimated by the Least Significance Difference (LSD) test at 1% level of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Determination of effect of the selected insecticides on soil microbial activity

Maximum amount of CO_2 (74.81 µg CO_2/g soil) was found from Marshal 20 EC treated soil followed by Admire 200 SL treated soil (65.89 µg CO_2/g soil) while minimum from untreated (Control) soil (37.57 µg CO_2/g soil) after 2 days of incubation (Table 1).

Admire 200 SL treated soil released maximum amount of CO_2 (126.5 µg CO_2/g soil) followed by Marshal 20 EC (110.9 µg CO_2/g soil) while minimum from Diazinon 60 EC (60.17 µg CO_2/g soil) at 4 days of incubation (Table 1).

Maximum amount of CO_2 (281.2 µg CO_2/g soil) was released from Marshal 20 EC treated soil followed by Admire 200 SL (162.1 µg CO_2/g soil), Dursban 20 EC (126.7 µg CO_2/g soil) and Diazinon 60 EC (118.6 µg CO_2/g soil) respectively while minimum from the untreated control (104.2 µg CO_2/g soil) at 8 days of incubation (Table 1).

Maximum CO_2 evolution was found from Admire 200 SL (222.4 µg CO_2/g soil) followed by Diazinon 60 EC (209.3 µg CO_2/g soil) while minimum from the control (163.7 µg CO_2/g soil) at 16 days of incubation (Table 1).

Maximum CO_2 evolution in soils was found from Marshal 20 EC treated soil (142.4 µg CO_2/g soil) followed by Admire 200 SL (133.4 µg CO_2/g soil) while minimum from control (59.4 µg CO_2/g soil) at 24 days of incubation (Table 1).

Several researchers reported the positive effect (Das *et al.* 1995; Bujin and Yongxi, 2000; Das and Mukherjee, 2000; Digrak and Kazanici, 2001), negative effect (Bhuiyan *et al.* 1992; Martinez-Toledo *et al.* 1995; Komal *et al.* 1999) or no effect (Komal *el al.* 1999; Iqbal *et al.* 2001) of different groups of insecticides during early stage of incubation on soil microorganisms. Bacterial population in Dimethoate treated plot was significantly less compared to those of the control plot after 2 days of incubation (Komal *et al.* 1999). Poor metabolism of chloropyriphos by soil microorganism and its negative impacts on non-target soil microorganisms was observed at early stage (Pozo *et al.* 1995) while adverse effect on fungi after 2, 4, and 6 weeks of treatments (Mallek *et al.* 1994). Dimethoate and Primicarb inhibited microbial respiration at high concentrations (Ahtiainen *et al.* 2003). Microbial respiration is mostly dependent upon the physiological condition of active microorganisms, nature and concentration of chemical as well as environmental conditions such as temperature; light etc. (Komal *et al.* 1999) also depends on the availability of organic matter or carbon sources. The amount of CO_2 evolution was higher in all insecticides treated soils than the control after 2 days of incubation which indicates the stimulatory effect of all insecticides on soil microorganisms during this period. Moreover, the increased amount of CO_2 evolution in Marshal 20 EC treated soils indicates the greater activity of soil. All the insecticides tested in the present study were useful in terms of microbial activity and microbial population. Marshal 20 EC having consistently higher positive stimulatory effect starting right from its application and continuing even up to 16 days of incubation. Similar but slightly less positive effect than Marshal 20 EC was observed in case of Admire 200 SL. Diazinon 60 EC and Dursban 20 EC had negative effect on microbial activity at 4 days of incubation but thereafter regained positive stimulation with time but was always less simulative than Admire 200 SL and Marshal 20 EC except at 16 days of incubation in case of Marshal 20 EC.

Determination of effect of the selected insecticides on soil Dehydrogenase activity

Maximum formazan formation was found in Diazinon 60 EC treated soil (0.073, 0.075, 0.079, 0.072 and 0.067 µmole/ml) while minimum from the control (0.038, 0.046, 0.059, 0.045 and 0.044 µmole/ml) at 2, 4, 8, 16 and 24 days of incubation respectively (Table 1).

The insecticides were useful in terms of dehydrogenase activity i.e. microbial population. Diazinon 60 EC had a consistent high positive dehydrogenase effect from starting incubation, continuing even up to 24 days of incubation. Similar but slightly less positive impacts were observed in Admire 200 SL, Marshal 20 EC and Dursban 20 EC compared to those of Diazinon 60 EC. The insecticides treated soils gave high formazan production than controlled soil. Maximum formazan production was obtained in the soil treated with Diazinon 60 EC. Temperatures lower than 37ºC were tested for the present study. Casida *et al.* (1977) previously showed that non-biological reduction of the TTC could occur at temperatures greater than this (37ºC) but the reaction rates were too slow as formazan production was lower. Nutrients become more readily available when dry soil is remoistened (Stevenson 1956). In addition, there is probably some breaking of dormancy for cells and depending on duration of incubation of remoistened soil there can be cellular multiplication. Substrates added to the soil for incubation trials usually are added as an aqueous solution so that the above aqueous response is a component of the response to the substrate. Regardless of weather a soil has been pre-incubated, dehydrogenase curves (formazan production) do not remain linear. Accumulations of metabolically generated CO_2 in soil might slow the metabolic rates of resident microorganisms. Dehydrogenase activity has been reported to be closely correlated with CO_2 release from soil (Skujins 1973). Soil dehydrogenase determinations conducted at 30ºC that 6-h incubation was more valid than longer incubations with a 1-h anaerobic incubation (Ross 1971).

Table 1. Effect of 4 selected insecticides on CO_2 evolution (µg/g soil) after different periods of incubation in the Laboratory [X]

Treatments	CO_2 evolution (µg/g soil) at different days of incubation									
	2		4		8		16		24	
Diazinon 60 EC @ 3.0 ml/L	43.9	d	60.2	e	118.6	d	209.3	b	104.0	c
Marshal 20 EC @ 2.0 ml/L	74.8	a	110.9	b	281.2	a	171.9	c	142.4	a
Dursban 20 EC @ 2.0 ml/L	52.1	c	75.7	d	126.7	c	163.7	d	89.3	d
Admire 200 SL @ 4.0 ml/L	65.9	b	126.5	a	162.1	b	222.4	a	133.4	b
Control	37.6	e	89.0	c	104.2	e	141.6	e	59.4	e
LSD (0.01)	2.0		2.0		2.6		2.9		1.8	
CV%	1.7		1.0		0.8		0.7		0.8	

[X] In a column means having the same letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.01 level of probability

Table 2. Effect of 4 selected insecticides on TPF obtained (µmole/ml) after different periods of incubation in the Laboratory [X]

Treatments	TPF obtained (µmole/ml) at different days after incubation									
	2		4		8		16		24	
Diazinon 60 EC @ 3.0 ml/l	0.073	a	0.075	a	0.079	a	0.072	a	0.067	a
Marshal 20 EC @ 2.0 ml/l	0.045	b	0.050	b	0.053	c	0.060	c	0.049	c
Dursban 20 EC @ 2.0 ml/l	0.030	d	0.048	c	0.037	d	0.034	e	0.031	e
Admire 200 SL @ 4.0 ml/l	0.044	b	0.048	c	0.051	c	0.063	b	0.056	b
Control	0.038	c	0.046	d	0.059	b	0.045	d	0.044	d
LSD (0.01)	0.018		0.002		0.014		0.004		0.003	
CV%	4.890		3.840		2.600		2.740		2.960	

[X] In a column means having the same letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.01 level of probability

CONCLUSION

Insecticides have a recognizable effect on the soil microbial activity as CO_2 evolution. In case of dehydrogenase activity, Diazinon 60 EC, Admire 200 SL and Marshal 20 EC gave greater positive effect than the control (untreated). In this experiment only the duration of incubation is considered but temperature varies in summer and winter drastically and soil become saturated in the monsoon in Bangladesh. As a result, the soil pH has changed. This is why further experiment is needed on the temperature, carbon sources and pH response on the microbial activity and microbial dehydrogenase activity along with the different insecticides.

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