THE EFFECT OF INSECTICIDES ON SOIL MICROORGANISMS

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CERTIFICATE

This is to certify that the thesis entitled **"THE EFFECT OF INSECTICIDES ON SOIL MICROORGANISMS"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN ARICULTURAL CHEMISTRY,*** embodies the result of a piece of *bona fide* research work carried out by **A. A. Masum Billah**, Registration No. **03-01133** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

I further certify that such help or sources of information, as have been availed during the course of this investigation have duly been acknowledged.

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Dedicated
to
My Beloved Parents

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LIST OF CONTENTS

CHAPTER	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	i
	LIST OF CONTENTS	ii
	LIST OF FIGURES	iv
	LIST OF APPENDICES	vi
	LIST OF ABBREVIATIONS	vii
	ABSTRACT	viii
I	INTRODUCTION	1-3
II	REVIEW OF LITERATURE	4-15
	2.1 Effect of insecticides on soil microorganisms	4
	2.2 Effect of Insecticides on soil Dehydrogenase Activity	11
III	MATERIALS AND METHODS	16-22
	3.1 Experimental Location	16
	3.2 Collection of Soil Sample	16
	3.3 Experiment 1. Determination of effect of the selected insecticides on soil microbial activity	16
	3.3.1 Pre-incubation of soil	16
	3.3.2 Experimental design and treatments	16
	3.3.3 Preparing the insecticidal solution	17
	3.3.4 Procedure for determination of soil microbial activity	17
	3.3.5 Microbial respiration	17
	3.4 Experiment 2. Determination of effect of the selected insecticides on soil Dehydrogenase activity	18
	3.4.1 Purpose	18
	3.4.2 Test characteristic	19
	3.4.3 Materials & Equipments	20
	3.4.4 Reagents	20
	3.4.4.1 Methanol and ethanol (AR Grade)	20
	3.4.4.2 Substrate solution (TTC), 1 % TTC solution	20

LIST OF CONTENTS (Continued)

CHAPTER	TITLE	PAGE NO.
	3.4.4.3 Glucose solution	20
	3.4.4.4 Triphenyl formazan (TPF) solution	21
	3.4.5 Procedure	21
	3.8 Statistical analysis	22
IV	RESULTS AND DISCUSSION	23-42
	4.1 Experiment 1. Determination of effect of the selected insecticides on soil microbial activity	23
	4.1.1 Incubation period: 2 days	23
	4.1.2 Incubation period: 4 days	23
	4.1.3 Incubation period: 8 days	26
	4.1.4 Incubation period: 16 days	26
	4.1.5 Incubation period: 24 days	26
	4.2 Experiment 2. Determination of effect of the selected insecticides on soil Dehydrogenase activity	33
	4.2.1 Incubation period: 2 days	33
	4.2.2 Incubation period: 4 days	33
	4.2.3 Incubation period: 8 days	36
	4.2.4 Incubation period: 16 days	36
	4.2.5 Incubation period: 24 days	36
V	SUMMARY AND CONCLUSION	43-45
	REFERENCES	46-51
	APPENDICES	52-53

LIST OF FIGURES

SERIAL NO.	TITLE OF THE FIGURES	PAGE NO.
Figure 1.	Hydrolysis of TTC to formazan	20
Figure 2.	Standard curve used for determination of the dehydrogenase activity in the tested soils	22
Figure 3.	Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 2 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	24
Figure 4.	Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 4 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	25
Figure 5.	Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 8 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	27
Figure 6.	Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 16 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	28
Figure 7.	Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 24 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	29

LIST OF FIGURES (Continued)

SERIAL NO.	TITLE OF THE FIGURES	PAGE NO.
Figure 8.	Trend of CO_2 evolution from 4 selected insecticides treated soils and untreated soil (control) at different time intervals of incubation in the laboratory.	31
Figure 9.	Comparative effects of 4 selected insecticides on dehydrogenase activity after 2 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	34
Figure 10.	Comparative effects of 4 selected insecticides on dehydrogenase activity after 4 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	35
Figure 11.	Comparative effects of 4 selected insecticides on dehydrogenase activity after 8 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	37
Figure 12	Comparative effects of 4 selected insecticides on dehydrogenase activity after 16 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	38
Figure 13.	Comparative effects of 4 selected insecticides on dehydrogenase activity after 24 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).	39
Figure 14.	Trend of dehydrogenase activity from 4 selected insecticides treated soils and untreated soil (control) at different time intervals of incubation in the laboratory.	41

LIST OF APPENDICES

APPENDICES	TITLE	PAGE NO.
Appendix 1.	Effect of 4 selected insecticides on CO_2 evolution (µg /g soil) after different periods of incubation in the Laboratory	52
Appendix 2.	Effect of 4 selected insecticides on TPF obtained (µmole/mL) after different periods of incubation in the Laboratory	53

LIST OF ABBREVIATIONS

%	=	Percent
@	=	At the rate
Anon.	=	Anonymous
DMRT	=	Duncan's Multiple Range Test
et al.	=	And Others
LSD	=	Least Significant Difference
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
ED	=	Ecological Dose
OD	=	Optical Dencities
DHA	=	Dehydrogenase Activity
EC	=	Emulcifiable Concentrate
η	=	Nano
μ	=	Micro

THE EFFECT OF INSECTICIDES ON SOIL MICROORGANISMS

ABSTRACT

BY

A.A. MASUM BILLAH

An experiment was conducted in the laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, during this period from July to December, 2009 to know the impact of 4 selected insecticide (Diazinon 60 EC, Marshal 20 EC, Dursban 20 EC and Admire 200 SL) on soil microorganisms and the impact was expressed as the amount of CO_2-C evolution per gram soil and dehydrogenase activity (TPF formation). The insecticides were injected in the soil samples and incubation. The data were taken after 2, 4,8,16 and 24 days of incubation for the determination of the microbial activity. The experiment revealed that all the insecticides tested had an effect on microbial activity or microbial population. The result shows that the amount of CO_2 is higher in all insecticide treated soils than that of control soil after 2 days of incubation, which indicates the stimulatory effect of all the insecticides on soil microorganisms during this period. Moreover, the high amount of CO_2 evolution in Marshal 20 EC treated soils indicates a grate activity of soil microorganisms. On the other hand Diazinon 60 EC and Durseban 20 EC showed negative effect on microbial activity till 4 days of incubation; but the microbial activity increased there after. In case of dehydrogenase activity, the insecticide treated soils gave higher formazan yields than that given by controlled soil. Diazinon 60 EC consistently high positive dehydrogenase effect starting right from its application and continued to have, even up to the last days of incubation. Similar but slightly less positive effect was observed in case of Admire 200 SL, Marshal 20 EC and Dursban 20 EC.

CHAPTER I

INTRODUCTION

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INTRODUCTION

Soil is a natural medium in which plants live, multiply and die and thus provides a perennial source of organic matter which could be recycled for plant nutrition. It is composed of five major components such as mineral matter, water, air, organic matter and living organisms. Among them, the last one makes up appreciably less than one per cent of the total soil volume (Purohit, 2003). The soil microorganisms can be categorized into bacteria, actinomycetes, fungi, algae and protozoa (Rao, 1995). The fertility of soil depends on its chemical composition, organic matter content and qualitative and quantitative nature of the soil microorganisms. These microorganisms have a major role in the metabolism of both organic and inorganic soil constituents for plants. Various groups of soil microorganisms decompose organic matter and most of the carbon is liberated as CO_2 during the decomposition. Therefore, the evolution of CO_2 serves as a measure of the rate and amount of the decomposition. The total amount of CO_2 liberated depends on the nature of the material, the microorganisms concerned and the conditions of the decomposition. Soil respiration is a good index of the activity of microorganisms involved in organic matter decomposition (Komal *et al.* 1999).

Synthetic insecticides are generally organic compounds and microorganisms are able to break down these compounds by their enzymes and utilize the carbon as a source of energy (Purohit, 2003). As a result, the population density of active microorganisms increases, which decomposes the more organic matter these is to liberate the more CO_2 into the atmosphere. 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). Dehydrogenases, as respiratory chain enzymes, play the major role in the energy production of organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme systems

of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in soil (Stevenson, 1956). Soils dehydrogenases are generally present in the upper layer of the soil. Concentration of soil dehydrogenases depends on conditions and intensity of biological conversion of organic compounds.

Non-optimal and non-judicious use of pesticides result in a series of problems related to both loss of their effectiveness in the long run and certain externalities such as pollution and health hazards (FAO, 2003). 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 the growth and activities of microorganisms in soil (Das and Mukherjee, 2000, Latif, 2007). On the other hand, some insecticides exerted adverse effect on the growth and activity of soil microorganisms (Susan *et al.* 2004, Ahtiainen *et al.* 2003, Digrak and Kazanici 200, Latif, 2007). But very little information is available on the effect of insecticides in soil microbial activities in Bangladesh.

As the insecticides applied in the soil, change the soil microbial activity and the soil microbial population affect the soil dehydrogenase activity. Lenhard (1965) introduced the concept of determining the metabolic activity of microorganisms in soil and other habitats by measuring dehydrogenase activity. A refinement of the Lenhard technique developed by Casida *et al.* (1977) has been widely used (Skujins, 1976). These studies have not resulted in uniformity of opinions as to which components of the soil ecosystem or the insecticides are contributing to the dehydrogenase response.

Rao (1995) revealed that insecticides in soil change the population of microorganisms. There are many reports regarding the favorable effects of insecticides on the growth and activities of microorganisms in soil (Das *et al.*

1995, Bujin and Yongxi 2000; Digrak and Kazanici, 2001). On the other hand, there are some insecticides, which exert adverse effect on the growth of soil microorganisms (Tu 1980, Bhuyan *et al.* 1992, Martinez-Toledo *et al.* 1995, Komal *et al.* 1999). Several reporters revealed that the effect of insecticides on soil microbial activities was temporary and it disappeared within a short period of time (Komal *et al.* 1999, Bujin and Yongxi 2000). However, no definite conclusion can be made on the effect of different insecticides on the growth and activities of microorganisms in soil, since different groups of insecticides exhibit manifold variations in toxicity (Komal *et al.* 1999, Das and Mukherjee 2000).

Therefore, the present study was under taken to know the effect of some selected insecticides on soil microorganisms. Accordingly, the experiment was conducted in the laboratory. The soil collected from the SAU field having treated selected insecticides with the following objectives:

- To investigate the impact of recommended doses of the selected insecticides on the CO_2 evolution by the soil microorganisms and
- To know the dehydrogenase activity of the soil as an indicate of soil microbial population.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Effect of insecticides on soil microorganisms

Furczak and Joniec (2007) conducted an experiment and the study was performed on a model of a field experiment in which a podzolic soil was fertilized with various doses of municipal-industrial sewage sludge (1, 2.5, 5, 10 and 20% of dry mass). Next, the soil was planted with willow (Salix viminalis L). After six months from the application of the sludge, determinations were made of the so-called total number of bacteria with low and high nutritional requirements, total number of fungi, number of cellulolytic and 'proteolytic' bacteria and fungi, respiratory activity, cellulose mineralization rate, intensity of ammonification, nitrification, dehydrogenases and protease activity in the soil. In the Ap horizon of the soil, higher doses of the sludge caused significant stimulation of growth of most of the studied groups of bacteria and fungi (with the exception of 'proteolytic' bacteria and fungi). Also, the stimulation of almost all of the biochemical parameters studied was observed, increasing with growing concentration of sludge. Only the process of ammonification was strongly inhibited in the treatment with 20% dose of sludge. In the deeper layer of the soil (20-40 cm) the effect of sewage sludge was weaker and less dependent on the dosage applied, than those effect in the Ap horizon. Only stimulation of growth of cellulolytic fungi was recorded and, in some treatments, of 'proteolytic' bacteria and fungi. Moreover, in most treatments significant increase was observed in the rate of respiration and cellulose mineralization. The study showed the existence of positive correlations among most of the studied microbial groups and biochemical properties of both soil horizons.

Latif (2007) tested the impact of 9 selected insecticides on soil microbial respiration and found stimulatory, inhibitory or no effects of insecticide treatments on soil microbial respiration and microbial activities. Flubendiamide. nimbicidine, lambdacyhalothrin, abamectin, and thiodicarb had stimulatory effect on microbial respiration during the initial period of incubation. Chloropyriphos, suntap and carbosulfan had inhibitory effect on microbial respiration and cypermethrin had no remarkable effect during the early stage of incubation. The negative effect of chloropyriphos, suntap and carbosulfan was temporary; it disappeared after 4 days of insecticides application. No effect of the selected insecticides on soil microorganisms was observed after 24 or 32 days of incubation.

Ingram *et al.* (2005) reported that diazinon and imidachloprid insecticides inhibited the growth of *Proteus vulgaris*, a urease-producing bacterium, but only diazinon significantly reduced urease activity in washed cells and insecticide inhibited urease activity in sonicated cells. Neither diazinon nor imidacloprid inhibited urease activity in Woolper soil (fine, mixed, mesic Typic Argiudoll) slurries. But diazinon slightly inhibited urease activity in Maury soil (fine, mixed, semiactive, mesic Typic Paleudalf) slurries. Imidacloprid had no effect on urease activity in creeping bentgrass or bluegrass sod at up to 10 times the commercial application rate. Diazinon briefly, but significantly reduced urease activity in bluegrass sod. Co-application of imidacloprid and urea appears to be benign with respect to urease activity in soil and sod. Diazinon, in contrast, appears to have a significant. short-term, inhibitory effect on the microbial urease producing community but that effect depends on soil type.

Xie-XiaoMei. *et. al.* (2004) conducted an experiment on the combined effects of nutrient and pesticide management on soil microbial activity in hybrid rice. Double annual cropping system were studied. The results of the field experiment demonstrated significant changes in soil microbial biomass, phospholipid contents, abundance of heterotrophic bacteria, proteolytic bacteria, electron transport

system/dehydrogenase activity and soil protein contents under different management practices and at various growth stages. Marked depletions in the soil microbial biomass and phospholipid contents were found with the advancement of crop growth stages, while the incorporation of fertilizers and/or pesticides also induced slight changes. The lowest microbial biomass and phospholipid content were found with the pesticide application alone. A decline in the bacterial abundance of heterotrophic bacteria and proteolytic bacteria was observed during the continuance of crop growth, while the lowest abundance of heterotrophic bacteria and proteolytic bacteria was found with the pesticide application alone, which coincided with the decline of soil microbial biomass. A consistent increase in the electron transport system activity was measured during the different crop growth stages of rice. The use of fertilizers (NPK) alone or combined with pesticides increased it, while a decline was noticed with pesticide application alone compared with the control. The soil protein content was found to be relatively stable with fertilizer and/or pesticide application at various growth stages in crops.

Susan *et al.* (2004) found that chloropyriphos was poorly metabolized by soil microorganisms, which resulted in accumulation and impacts on non-target soil microorganisms and fauna. Its application also significantly reduced root production, tardigrade and nematode populations and altered microbial community structure and function.

Ahtiainen *et al.* (2003) assessed the effects of dimethoate and pirimicarb on soil microorganisms both in the field and the laboratory. In the field studies, significant effects of pesticide treatments on microbial processes were not observed. In the laboratory studies the toxicity of certain pesticides was clearly detected by bacterial toxicity tests. However, in the soil respiration inhibition assessment with soil similar to that used in the field trial, inhibition was observed only at unrealistically high concentrations. Similarly, Roger and Coderre (1995) reported that deltamethrin and primicarb had no consistent negative effect on bacterial

activity. The bacterial activity was, however, affected positively by primicarb.

Iqbal *et al.* (2001) conducted a field experiment the result of which revealed that othofonprox, profenophos + cypermethrin and bifenthrin + endosulfan inhibited, while endosulfan, imidachlopid, methamidophos, endosulfan along with dimethoate, profenofos + alphmethrin, chloropyriphos + tralomethrin + acetamprid and cyhalothrin + profenofos + diafenthiuron, stimulated nitrification in soil. All other pesticidal applications had no effect on this parameter. Samples collected at different intervals of time from all the fields in three years study showed no differences in nitrification from sowing to harvest. The variations observed, in general, being very week and transient and resulting in a recovery of nitrification.

Digrak and Kazinici (2001) reported the effect of some organophophorous insecticides such as isofonofos, phorate and fonfos on soil microbiota. Number of the total available bacteria was found to be higher in the isofonofos treated soil sample than that of the control groups, during incubation period. Moreover, it was this insecticide, which had no inhibitory effect on the development of the other microorganism groups. It was determined that the fonfos and the phorate were had no inhibitory effect on the development of the soil microorganism.

Das *et al.* (1995); Bujin and Yongxi, (2000), conducted different experiments and found that soil microorganisms are scavengers in soil. They degrade a variety of chemical substances including insecticides in soil to derive energy and nutrients for their growth and development. They play a primary catabolic role in the environment through degradation of plant and animal residues. Insecticides are frequently used in the field to increase crop production. The continuous and repeated use of insecticides has some effects on soil microbial activities such as the population of soil microorganisms, soil respiration, nitrogen fixation, ammonification, nitrification and enzyme activities in soil. Some insecticides favored the growth and activities of microorganisms in soil.

Bhuyan *et al.* (1992); Martinez-Toledo *et at.* (1995), found that some insecticides exerted adverse effect on the growth of the soil microorganisms.

Das and Mukherjee (2000) investigated the effect of four insecticides viz. BHC phorate, carbofuran, and fenvalerate on the growth and activities of N_2 fixing and phosphate- solubilizing microorganisms in relation to the availability of N and P in laterite soil. Insecticides in general and BHC and phorate in particular, stimulated the proliferation of aerobic non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms and also enhanced their biochemical activities, such as non-symbiotic N_2-fixing and phosphate-solubilizing capacities, which resulted in greater release of available N (NH_4^+ and NO_3^-) and P in soil. All the insecticides were persistent in soil for a short period of time, and the rate of dissipation was highest for fenvalerate followed by phorate, carbofuran, and BHC, depicting the half lives ($T_{1/2}$) 8.8, 9.7, 16.9, and 20.6 days, respectively. The insecticides followed first-order reaction kinetics during their dissipation in soil.

Komal *et al.* (1999) observed all of the stimulatory, inhibitory or no effects of insecticide on CO_2 evolution. There was statistically significant decrease in soil respiration 15 days after monocrotophos treatment, 20 days after dimethoate treatment and 10 days after triazophos treatment. The degree of effect depends on the chemical substances, dosages of chemicals and period after insecticide application. The insecticides used had only temporary effects on microbes and microbial activities, which disappeared either before the next insecticide treatment or at the end of the experiment.

Pozo *et al.* (1995) studied the effects of the insecticide chloropyriphos on soil microbial activity. The presence of 2.0, 3.5, 5.0, and 10.0 kg/ha of chloropyriphos significantly ($p<0.05$) decreased aerobic dinitrogen-fixing bacteria and dinitrogen fixation, particularly after a second insecticide treatment. The total number of bacteria increased significantly at concentrations of 2.0 to 10.0 kg/ha. Activities of

acid and alkaline phosphatases and dehydrogenase significantly decreased initially at concentrations of 2.0 to 10.0 kg/ha, but recovered after 14 days to levels similar to those in control soil without chloropyriphos. Fungal populations, nitrifying bacteria, and denitrifying bacteria were not affected as a consequence of the addition of the insecticide.

Mallek *et al.* (1994) tested the effects of profenfos on fungal populations and some activities in soil. Profenfos (at 5.4 micrograms active ingredient/g dry soil), had a significant adverse effect on the count of total fungi after 2, 4 and 6 weeks after treatment. This effect was completely alleviated after longer incubation. Incorporation of this insecticide into the agar medium inhibited the total count of soil fungi at 6.4 and 38.4 micrograms/mL. Initial activation followed by a decrease in CO_2 output occurred in soil treated with 5.4 micrograms a.i./g. The two doses of profenfos accelerated urease activity for 6 weeks after soil treatment. but inhibited the enzyme activity after longer periods. An inhibitory effect on nitrate reductase activity was observed with some insecticide treatments in the early stages of incubation followed by activation in certain cases.

Rangaswamy and Venkateswarlu (1993) reported that mineralization of peptone-nitrogen and oxidations of ammonical nitrogen were significantly enhanced in two agricultural soils treated with cypermethrin and fenvalerate up to 5 kg/ha. The rate of ammonification and nitrification after 2 and 4 weeks was fairly rapid in the soils receiving 2.5 kg/ha of either insecticide. The enhancement of both transformations mediated by microorganisms was more pronounced in the fenvalerate-treated soils. Cultures of *Azospirillum* sp., isolated from insecticide treated soils, exhibited greater nitrogen fixing activity that lasted for at least three generations.

Gonzalez-Lopez *et al.* (1992) reported that the presence of 10–300 μg/g of methidathion significantly increased fungal populations (colony-forming units). Denitrifying bacteria, aerobic N_2-fixing bacteria and N_2 fixation were significantly increased at concentrations of 50–300 μg/g. The total number of bacteria increased

significantly at concentrations of 100–300 µg/g. Nitrifying bacteria decreased initially at concentrations of 300 µg/g, but recovered rapidly to levels similar to those in the control soil without the insecticide concentrations of 300 µg/g, but recovered rapidly to levels similar to those in the control soil without the insecticide.

Belanger *et al.* (1982) reported that humus enhanced the persistence of fonofos and curtailed the stimulating effect of fonofos on soil microbial populations.

Newell *et al.* (1981) determined the effects of 24 to 72 hr exposure to fenthion [10(1)-10(3) ppb] for a fungal community, nitrogen-fixing microbes, and representative micro faunal and zooplankton invertebrates of a mangrove ecosystem and also the abilities of a benthic diatom and of fungi to grow in the presence of fenthion. Acute lethal, growth-inhibiting, or process-disrupting effects were not detected for exposures to less than 500 ppb fenthion.

Tu (1980) conducted a laboratory test with five pyrethroid insecticides permethrin (FMC 33297), FMC 45498, shell WL 41706, shell WL 43467 and shell WL 43775 at 0.5 and 5 µg/g and revealed that insecticides had antimicrobial activity in early stages of incubation. The populations recovered after 2 to 4 weeks and stimulatory effects on populations were also observed in later stages. Soil microbial respiration, as indicated by oxygen consumption, increased with increasing concentration of insecticides, suggesting the possibility of microbial degradation of the insecticides.

Tu and Mile (1976) reported that excessive concentration of the insecticides in soil may shift microbial populations to temporarily favored group(s) of microorganisms, which might overpopulate the soil. Beneficial microorganisms could be suppressed provoking new and more complex problems from microorganisms, which had escaped competition with other suppressed groups.

2.2. Effect of Insecticides on Soil Dehydrogenase Activity

Srivastava, P.K. (2009) conducted an experiment and found that forest tree growth in mid altitude of Central Himalayan ecosystem is most commonly limited by shortage of mineral nutrients. Consequently, in these circumstances growth depends on nutrient cycling in the soil in rhizosphere region specifically due to presence of microbes that transfer soil organic matter and release mineral nutrients into forms available to plants. Rapid replacement of oak oriented forest with pine oriented forest in Garhwal for its commercial product or non-timber forest product cause a huge loss of various ecological attributes that are associated with oak forest soil. In Mid Altitude Central Himalaya, it has been found that, in many aspects, oak oriented forest is better than pine oriented forest, whether it is on the basis of nutrient status or microbial activity. Continuous deforestation of oak oriented forest causes reduction in microbial activity in the soil. Prediction of microbial activity can be done by estimation of dehydrogenase activity in the soil. The mean dehydrogenase activity (DHA) obtained in oak forest soil were 106.28 m mol /g dry weight soil/ 2 hr and for pine forest it was 66.37 m mol /g dry weight soil/ 2 hr respectively, indicate a higher microbial activity in oak oriented forest than pine oriented forest.

Ghaly and Mahmoud (2006) conducted an experiment and found that the suitability of the triphenyl tetrazolium chloride (TTC) for dehydrogenase activity measurement of the vegetative cells of the fungal species *Aspergillus niger* was investigated. The triphenyl formazan (TF) yield increased with the increases in TTC concentration, pH, temperature and incubation time with in the studied ranges. The effects of individual parameters, as well as the combined effects, on the TF yield were found to follow exponential expressions. The sensitivity analysis showed that the TF yield is more sensitive to change in temperature followed by pH, TTC concentration and incubation time. Although the rate of increase in the enzyme activity decreased gradually with the increase in

temperature, no enzyme denaturation was observed below 55^0 C.

Stepniewska and Wolinska (2003) conducted an experiment and found that the paper presents the influence of chromium forms (III) and (VI) on the soil dehydrogenase activity. Enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. It was found that chromium compounds have detrimental effect on soil dehydrogenase activity. After the addition of chromium, a rapid and significant decrease in enzymatic activities was observed.

Moreno *et.al.* (2002) an experiment was conducted and found that used biochemical measures related with the soil microbial activity to evaluate the toxic effects of Cd in two semiarid soils of different characteristics, which have been submitted to different management practices. The Cd concentrations which produced 5, 10 and 50% inhibition of the parameter studied (Ecological Dose values) were calculated after incubation periods of 3 hours, 20 days and 60 days. In addition, *Lolium perenne* was cultivated in both soils containing the different Cd concentrations in order to ascertain the effect of Cd contamination on plant growth. When the dehydrogenase activity and ATP content of the soils were analysed, the calculated Ecological Dose values (EDV) were higher in the agricultural soil than in the abandoned soil. The yield of *Lolium perenne* fell gradually with increasing concentrations of Cd in the soil, the lowest decrease being observed in the agricultural soil at 60 days. The concentration of Cd in the plants increased gradually with the total and DTPA-extractable concentration of this heavy metal in the soil.

Jeanette M. Norton and Mary K. Firestone (1991) conducted an experiment to find the quantity and metabolic status of bacteria and fungi in rhizosphere and nonrhizosphere soil from microcosms containing ponderosa pine seedlings. Rhizosphere soil was sampled from adjacent to coarse, fine, or young roots. The

biovolume and metabolic status of bacterial and fungal cells was determined microscopically and converted to total and active biomass values. Cells were considered active if they possessed the ability to reduce the artificial electron acceptor 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) to visible intracellular deposits of INT formazan. A colorimetric assay of INT formazan production was also used to assess dehydrogenase activity. INT-active microorganisms made up 44 to 55% of the microbial biomass in the soils studied. The proportion of fungal biomass that exhibited INT-reducing activity (40 to 50%) was higher than previous estimates of the active proportion of soil fungi determined by using fluorescein diacetate. Comparison between soils from different root zones revealed that the highest total and INT-active fungal biomass was adjacent to fine mycorrhizal roots, whereas the highest total and active bacterial biomass was adjacent to the young growing root tips. These observations suggest that fungi are enhanced adjacent to the fine roots compared with the nonrhizosphere soil, whereas bacteria are more responsive than fungi to labile carbon inputs in the young root zone. Colorimetric dehydrogenase assays detected gross differences between bulk and rhizosphere soil activity but were unable to detect more subtle differences due to root types. Determination of total and INT-active biomass has increased our understanding of the role of spatial compartmentalization of bacteria and fungi in rhizosphere carbon flow.

Casida (1977) conducted an experiment on the dehydrogenase technique for measuring the metabolic activity of microorganisms in soil with modification to use a 6-h, 37°C incubation with either glucose or yeast extract as the electron-donating substrate. The rate of formazan production remained constant during this time interval, and cellular multiplication apparently did not occur. The technique was used to follow changes in the overall metabolic activities of microorganisms in soil undergoing incubation with a limiting concentration of added nutrient. The sequence of events was similar to that obtained by using the Warburg respirometer to measure O_2 consumption. However, the major peaks of activity occurred earlier

with the respirometer. This possibly is due to the lack of atmospheric CO_2 during the O_2 consumption measurements.

Skujins (1973) investigated and considered alteration in buffer systems, pH values, amounts of TTC used, and incubation period; the effect of O_2; the lack of added substrate (actual dehydrogenase activity) versus use of glucose, glucose 6-phosphate, DL-alanine, etc. (potential dehydrogenase activity); nicotinamide adenine dinucleotide additions; preliminary leaching of the soil; and additions of toluene, chloroform, chloramphenicol, and KCN. Comparisons of respective dehydrogenase activities have been made for variations in microbial numbers, oxygen consumption, CO_2 evolution, soil C:N ratios, soil humus content, other enzymes in soil, plant growth and yields, soil particle size fractions, soil sampling depth, season, and so forth. These studies have not resulted in a uniformity of opinion as to which components of the soil ecosystem are contributing to the dehydrogenase response.

Klein *et al.* (1971) incubated soil for 96 h with glucose at 27°C, and Skujins (1973) incubated soil for 24 h at 30°C without added substrate. Ross (1971) incubated soil for 1 h anaerobically or 6 h aerobically with glucose at 30°C. The 24-h (or longer) incubation period seems to be the part of the dehydrogenase technique, most likely to cause problems in the interpretation of results. For example, a uniform rate of formazan production should occur during this time and cellular multiplication, particularly enriching for certain components of the microbial population should not occur.

Lenhard (1965) introduced the concept of determining the metabolic activity of microorganisms in soil and other habitats by measuring dehydrogenase activity. In general, the technique involves the incubation of soil with 2, 3, 5-triphenyltetrazolium chloride (TTC) either in the presence or absence of added electron-donating substrates. Microbial dehydrogenase activity during this incubation, results in reduction of the water soluble, colorless TTC to the water-

insoluble red 2, 3, 5-triphenyltetrazolium formazan. The latter is then extracted from the soil and read colorimetrically for quantization. A refinement of the Lenhard technique by Casida *et al.* (1977) has been widely used. Many variations on the technique have now been tried, and the results have been interpreted in various ways by various workers.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Location

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.

3.2 Collection of Soil Sample

The soil used in the experiment was collected from the farm of SAU, Dhaka, Bangladesh. The collected soil was clay loom to loam in texture and acidic in pH of around 5.8 and of moderate fertility status. Soil (0-15 cm) was brought to the laboratory, 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, the soil was pre-incubated in the laboratory.

3.3 Experiment 1. Determination of effect of the selected insecticides on soil microbial activity

3.3.1 Pre-incubation of soil

The soil with 40% water holding capacity was subjected to pre-incubation aerobically at room temperature for 10 days. Pre-incubation was performed in a plastic container. 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.

3.3.2 Experimental design and treatments

The experiment was laid out in Completely Randomized Design (CRD) with three replications. There were five 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 (details of the insecticides used and their doses are given in Appendix 1) and a control.

3.3.3 Preparing the insecticidal solution

Insecticide solution was prepared by taking exactly required of each insecticide in 1 liter volumetric flask marked for each insecticide, and 200 ml of distilled water was added to each. The flasks were then shaken for five minutes for proper dilution of insecticides with water. After shaking, the volume was made up to the mark by adding more distilled water. Only one liter distilled water was taken in a flask marked for control.

3.3.4 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 except control. Five milliliters of distilled water was added to the control soil to maintain moisture content equivalent to those of treated soils. Following insecticide applicated 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.

3.3.5 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. $NaOH + CO_2 \rightarrow Na_2CO_3 + NaHCO_3 + H_2O$ pH = 12
2. $Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl$ pH = 12- 8.3
3. $NaHCO_3 + HCl \rightarrow H_2CO_3 + NaCl$ 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 = figure conversion
w = weight of soil in g (60 g soil)

3.4 Experiment 2. Determination of effect of the selected insecticides on soil Dehydrogenase activity

3.4.1 Purpose

The test was designed for the determination of microbial activation of soils and direct effect of pesticides. Since the toxicity influences several bacterial generations it can be considered as subchronical exposition. Soil activation is determined indirectly, using hydrolytic reaction leading to formazan production.The formazan concentration is directly proportional to the vitality level of the community of soil nonphotosynthetic microorganisms. The activation rate can be expressed as activity of soil dehydrogenases or relatively in percentage in comparison to control.

3.4.2 Test characteristic

Dehydrogenases are generally present in the upper layer of every soils. Soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulphur and phosphorus cycles. Dehydrogenases, as respiratory chain enzymes, play the major role in the energy production of organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme systems of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in soil. However, soil dehydrogenases come from activities of plants and soil microorganisms, as well. Concentration of soil dehydrogenases depends on conditions and intensity of biological conversion of organic compounds. Addition of suitable chemical i.e. triphenyltetrazolium chloride enhances bioavability of endogenous soil organic compounds to microflora. At the same time the chloride is converged by hydrolytic reaction to formazan which can be extracted by organic solvents (methanol, acetone). Formazan concentration can be determined spectrophotometrically at 485 ηm. Addition of organic substrate, e.g. compost, induces maximum DHA. Actual hydrolytic reaction is shown on Fig.1.

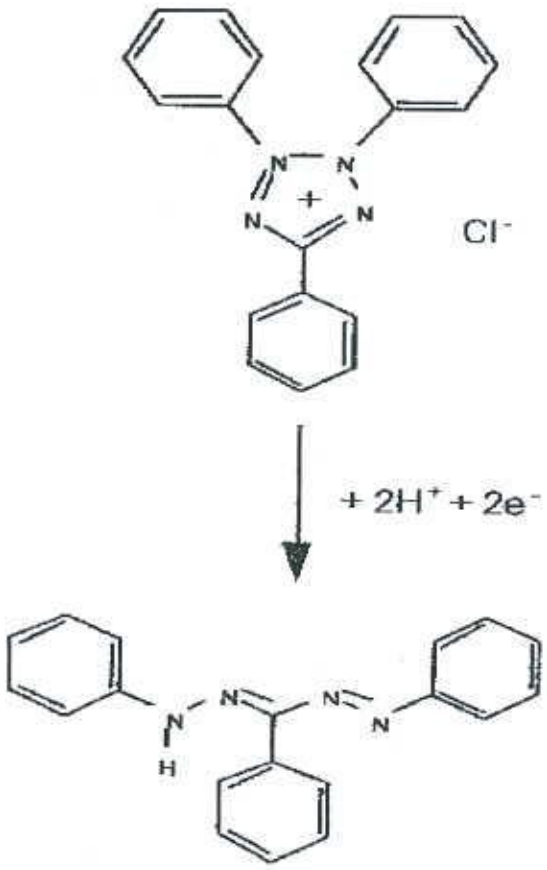

Figure 1. Hydrolysis of TTC to formazan

3.4.3 Materials & Equipments

Air tight screw cap test tube, Incubator, Spectrophotometer

3.4.4 Reagents

3.4.4.1 Methanol and ethanol (AR Grade)

3.4.4.2 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 4 °C in dark or in amber bottle.

3.4.4.3 Glucose solution

For preparing glucose solution 1 g of glucose was dissolved in 100 mL distilled water.

3.4.4.4 Triphenyl formazan (TPF) solutions

A standard solution of 0.0002 M triphenyl formazan ($C_6H_5N:NC[C_6H_5]:NNHC_6H_5$) was prepared by dissolving 0.03 g triphenyl formazan in 500 mL ethyl alchol.

3.4.5 Procedure

1. 1 g air dried representative soil sample was taken in an air tight screw capped test tube (15 mL capacity).
2. 0.2 mL of 3% TTC solution was added to each of the tubes to saturate the soil.
3. 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.
4. The tubes were incubate at 28±0.5 °C for 24 hours.
5. After incubation, 10 mL of methanol was added, shaken vigorously and with stand for 6 hours.
6. The clear pink coloured supernatant fluid was removed and the reading was taken with a spectrometer at wave length of 485 ηm.
7. The amount of TPF formed was calculated from the standard curve.

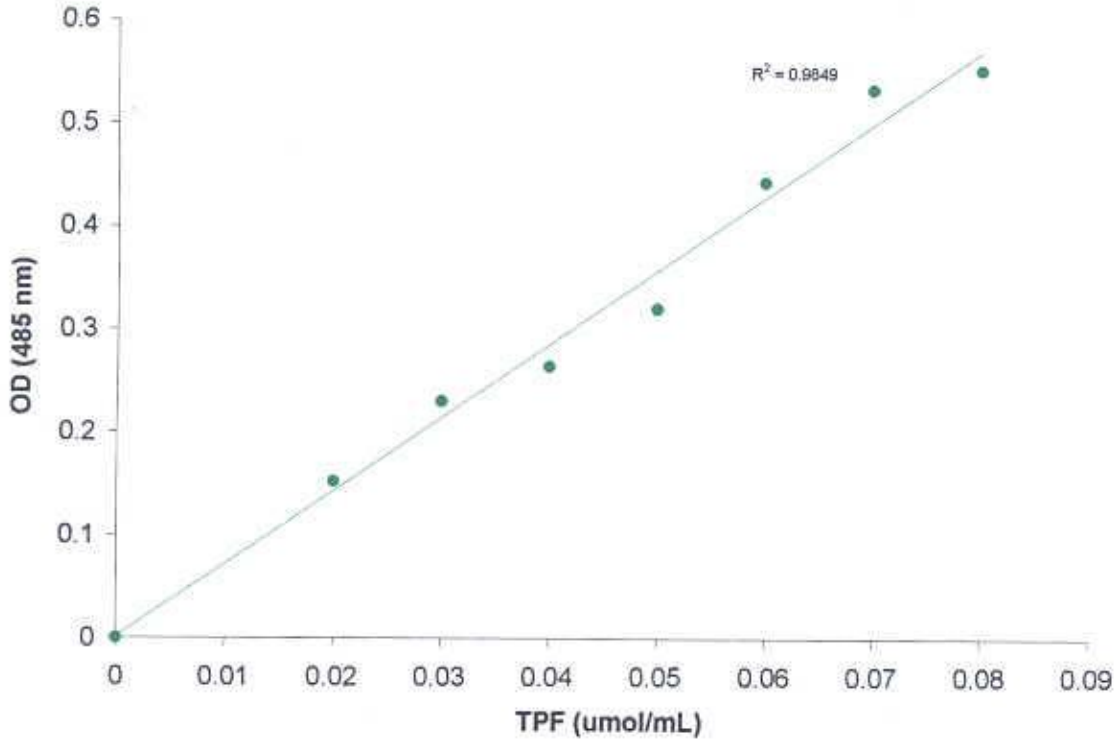

Figure 2. Standard curve used for determination of the dehydrogenase activity in the tested soils

3.8 Statistical analysis

The computer using statistical package program MSTAT-C developed by Russel (1986) is used to analyze the collected data. ANOVA was made by F variance test and the pair comparisons were performed by Duncan multiple range test (DMRT) (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Experiment 1. Determination of effect of the selected insecticides on soil microbial activity

The impact of 4 selected insecticides on soil microorganisms was determined in terms of the amount of CO_2 evolution per gram soil during the decomposition of organic matter by soil microorganisms after 2, 4, 8, 16 and 24 days of incubation. The results are presented in Figure 3 to 8 and Appendix 1.

4.1.1 Incubation period: 2 days

Figure 3 indicates that the highest amount of CO_2 (74.81 µg CO_2 /g soil) was recorded from Marshal 20 EC treated soil followed by Admire 200 SL treated soil (65.89 µg CO_2 /g soil) after 2 days of incubation. The lowest amount of CO_2 (37.57 µg CO_2 /g soil) was recorded from the untreated (Control) soil.

4.1.2 Incubation period: 4 days

The amount of CO_2 evolution after 4 days of incubation presented in Figure 4 indicates that the Admire 200 SL treated soil released the greatest amount of CO_2 (126.5 µg CO_2 /g soil), which was significantly different from other insecticide treated soils. The amounts of CO_2 recorded from Marshal 20 EC (110.9 µg CO_2/g soil) were significantly higher than that of the control soil (Appendix 1). But Diazinon 60 EC treated soil released 60.17 µg CO_2/g soil, which was significantly lower than that of the control soil.

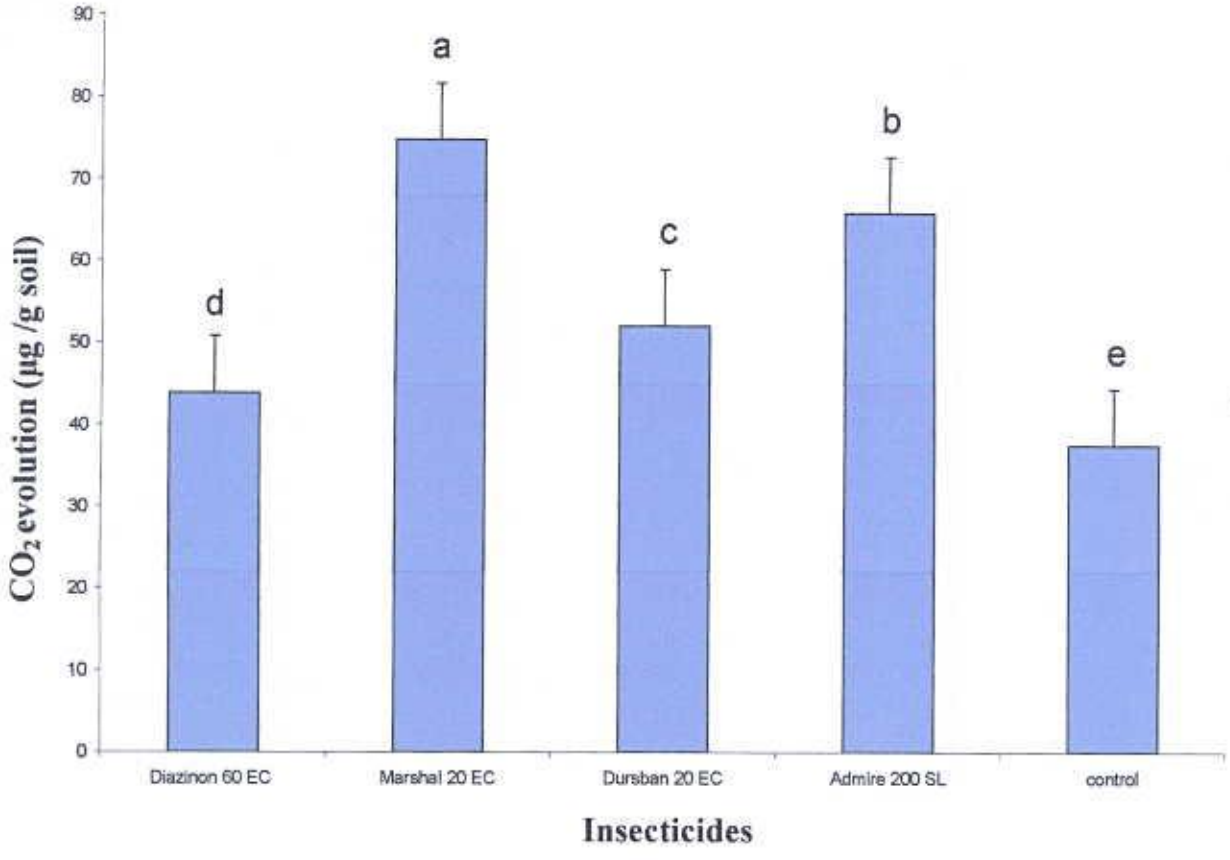

Figure 3. Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 2 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

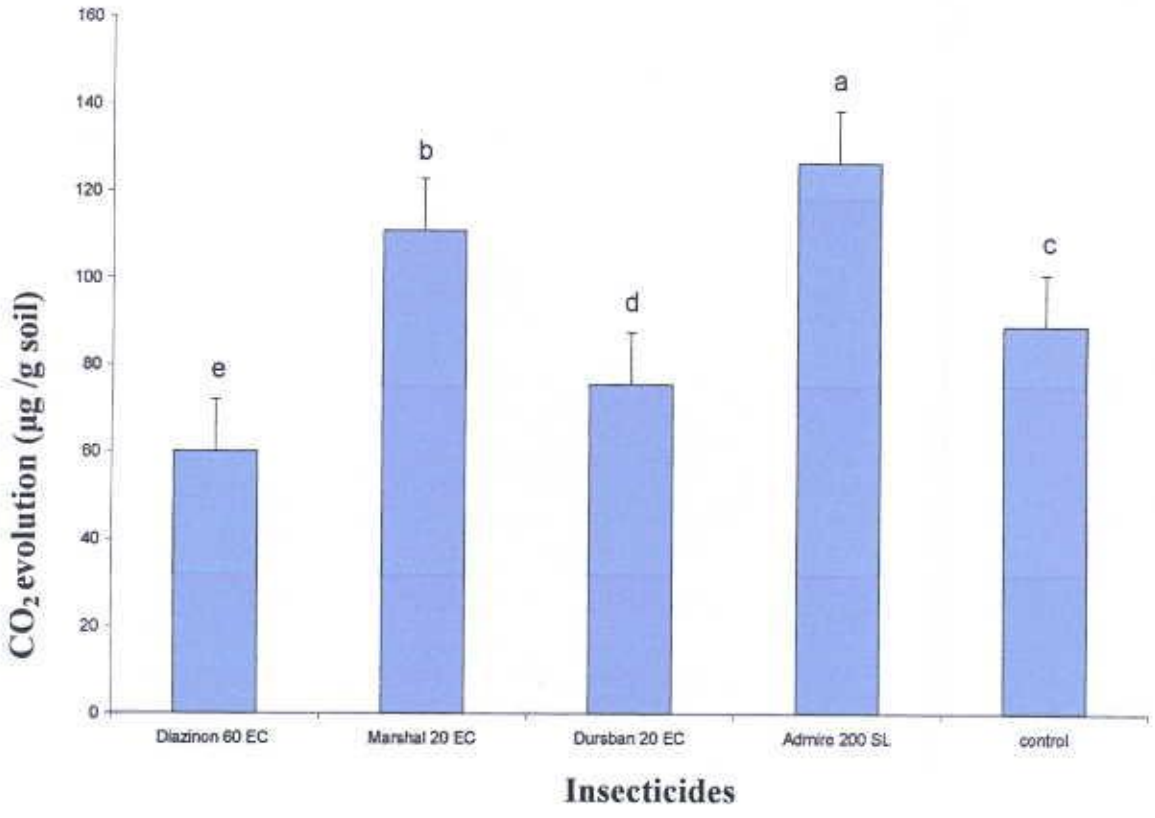

Figure 4. Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 4 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

4.1.3 Incubation period: 8 days

After 8 days of incubation the highest amount of CO_2 (281.2 µg CO_2/g soil) was released from Marshal 20 EC treated soil, which was much higher than all other insecticides treated soil (Figure 5). The amount of CO_2 released from Admire 200 SL Dursban 20 EC and Diazinon 60 EC was 162.1 µg CO_2/g soil, 126.7 µg CO_2/g soil and 118.6 µg CO_2/g soil, respectively. While the lowest amount of CO_2 (104.2 µg CO_2/g soil) release was observed in control soil.

4.1.4 Incubation period: 16 days

The amount of CO_2 evolution was 222.4 µg CO_2/g soil from Admire 200 SL treated soil after 16 days of incubation, which was much higher than that of any other insecticides treated soils as shown in Figure 6. Diazinon 60 EC, Marshal 20 EC and Dursban 20 EC treated soil released 209.3, 171.3 and 163.7 µg CO_2/g soil respectively, which was significantly greater than control soil (Appendix 1).

4.1.5 Incubation period: 24 days

The results presented in the figure 7 shows the CO_2 evolution in soils that after 24 days of incubation. The highest amount of CO_2 (142.4 µg CO_2/g soil) was obtained in Marshal 20 EC treated soil followed by Admire 200 SL (133.4 µg CO_2/g soil). Diazinon 60 EC (103.98 µg CO_2/g soil) and Dursban 20 EC (89.32 µg CO_2/g soil) treated soils released significantly higher amount of CO_2 than that of control. The lowest amount (59.41 µg CO_2/g soil) of CO_2 was released from untreated soil (Appendix 1).

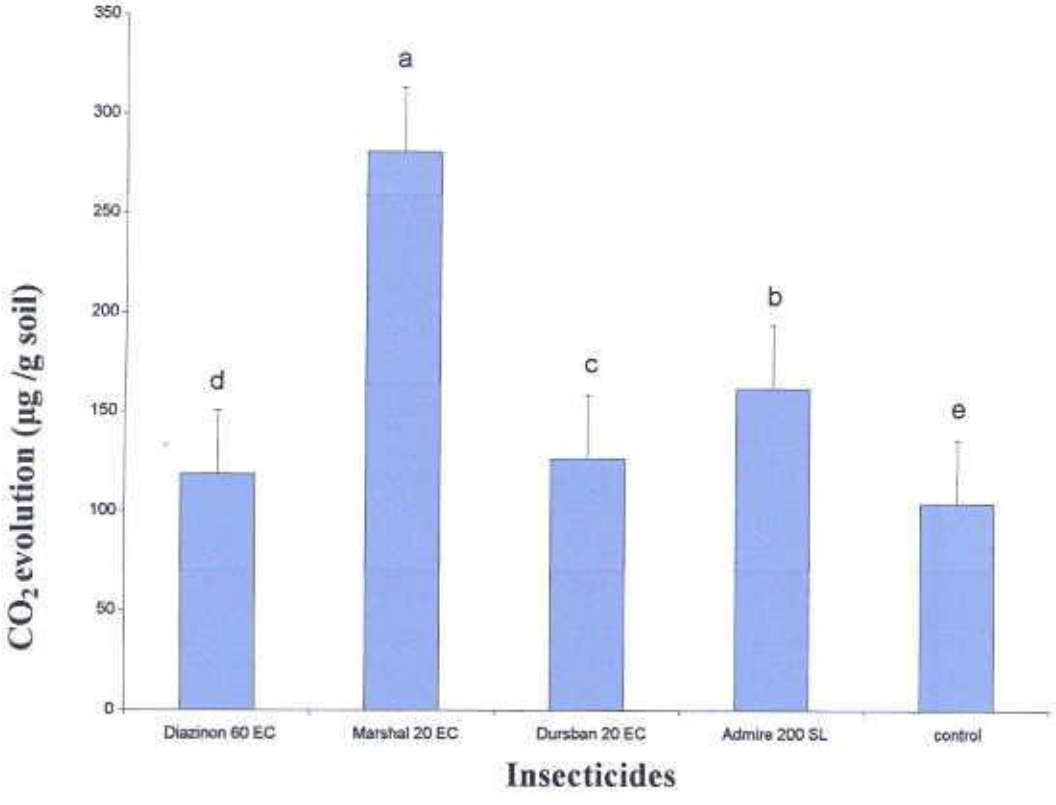

Figure 5. Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 8 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

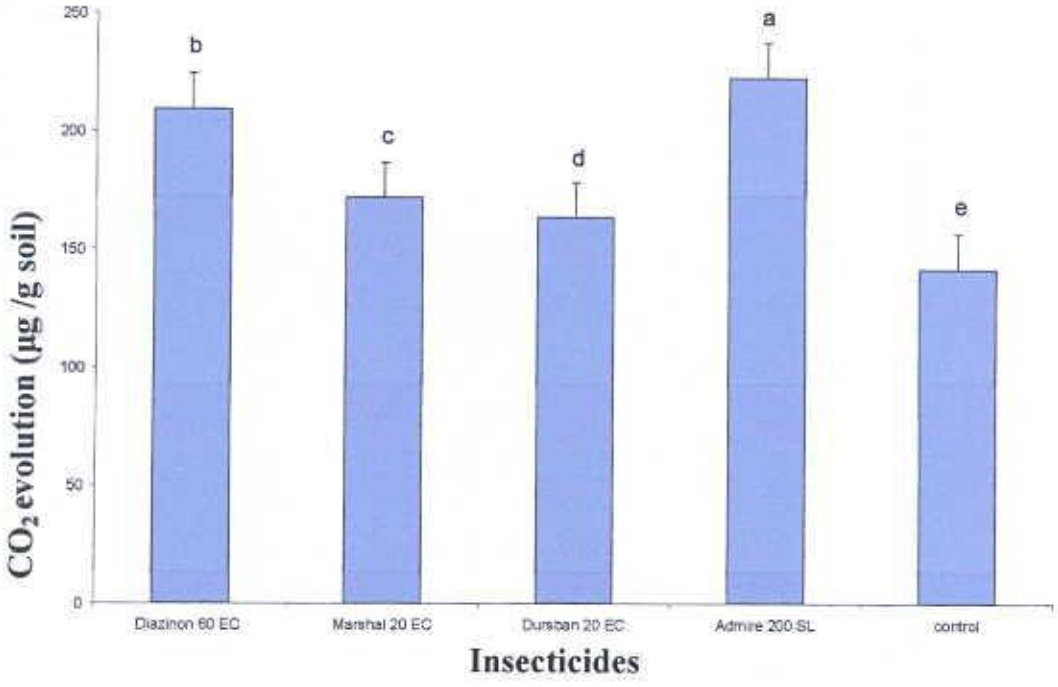

Figure 6. Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil)after 16 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test).The T shaped beams represent the standard errors).

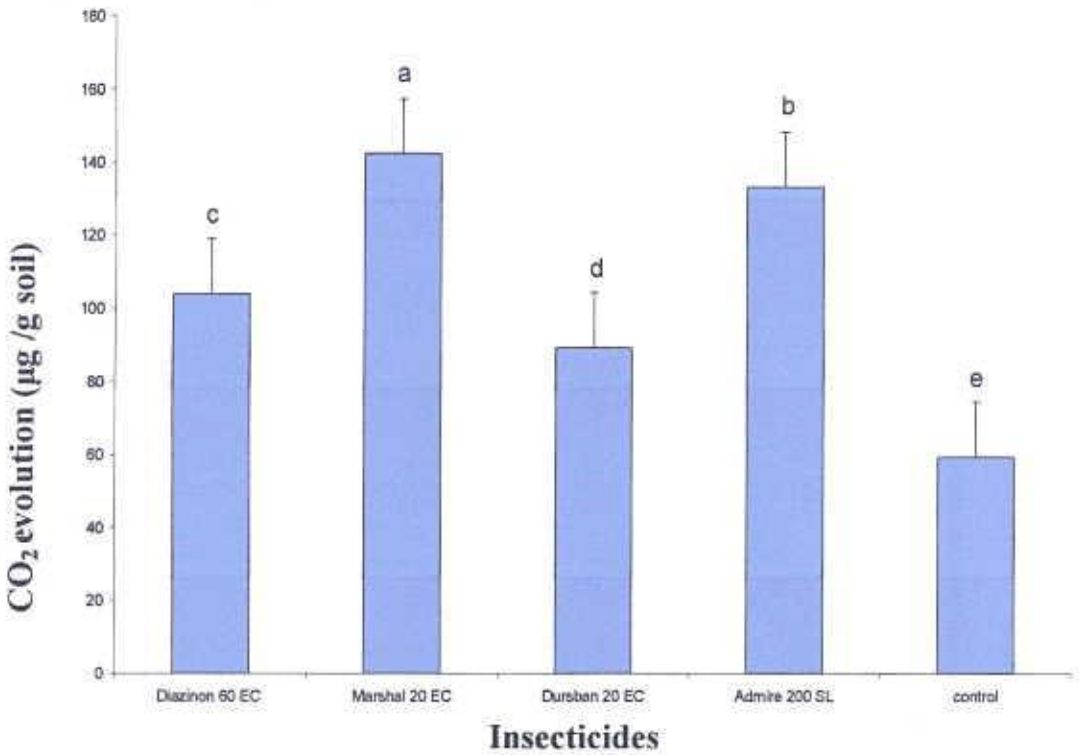

Figure 7. Comparative effects of 4 selected insecticides on CO_2-C evolution (µg/g soil) after 24 days of insecticides application in the laboratory. (Values having different letters are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

Several researchers reported the positive effect of different groups of insecticides during early stage of incubation (Das *et al.* 1995, Bujin and Yongxi 2000, Das and Mukherjee 2000, Digrak and Kazanici 2001). On the other hand, some researchers observed negative (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 insecticides on soil microorganisms. Komal *et al.* (1999) observed that bacterial population in the dimethoate treated plot was significantly less compared to control after 2 days of incubation. Poor metabolism of chloropyriphos by soil microorganism and its negative impacts on non-target soil microorganisms at early stage was observed by Pozo *et al.* (1995) and Susan *et al.* (2004). On the other hand, *Mallek et al.* (1994) observed the adverse effect on fungi after 2, 4, and 6 weeks of treatments. The similar result also observed by Ahtiainen *et al.*, (2003) and they revealed that dimethoate and primicarb inhibited microbial respiration at high concentrations. The results however may differ from that of the other researchers but logical because the microbial respiration is mostly dependent upon the physiological condition of the active microorganisms, the nature and concentration of the chemical as well as the environmental conditions such as temperature, light etc. (Komal *et al.* 1999). It also depends on the availability of the organic matter or carbon sources.

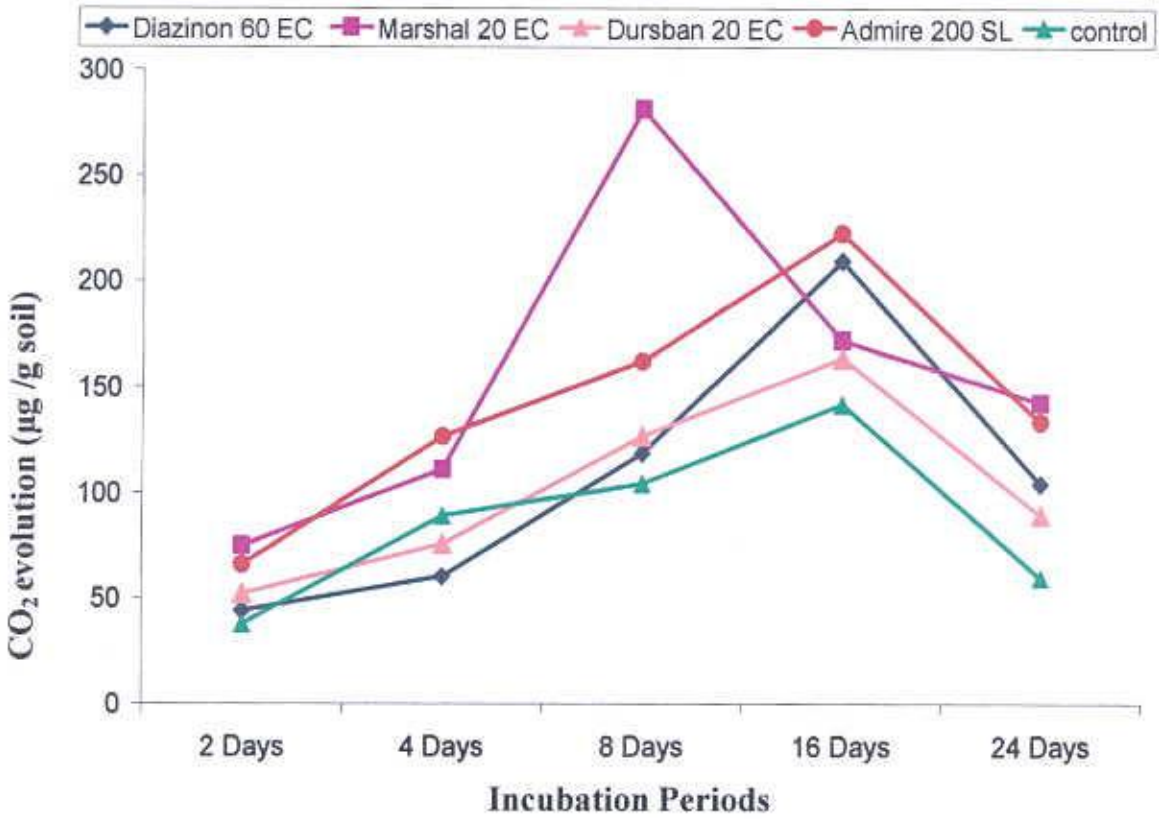

Figure 8. Trend of CO_2 evolution from 4 selected insecticides treated soils and untreated soil (control) at different time intervals of incubation in the laboratory.

The amount of CO_2 evolution is higher in all insecticides treated soils than control soil 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.

The experiment thus revealed that 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 there after 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.

4.2 Experiment 2. Determination of effect of the selected insecticides on soil Dehydrogenase activity

Dehydrogenase values were determined for tested soils after they had incubated for 2, 4, 8, 16 and 24 days with TTC. These soils produced highest levels of formazan than that of soil not receiving different insecticides. Dehydrogenase values at different time intervals of incubation were shown in Figure 9 to 14 and Appendix 2.

4.2.1 Incubation period: 2 days

The results presented in the figure 9 shows that after 2 days of incubation formazan formation was highest in Diazinon 60 EC treated soil (0.073 µmole/mL) which was much higher than all other insecticides treated soil (Figure 9). Marshal 20 EC and Admire 200 SL treated soil released 0.045 µmole/mL and 0.044 µmole/mL formazan respectively, which was statistically similar and significantly greater than control soil (0.038 µmole/mL) (Appendix 2). The lowest amount (0.030 µmole/mL) of Formazan was released from Dursban 20 EC treated soil. (Appendix 2).

4.2.2 Incubation period: 4 days

The amount of Formazan obtained after 4 days of incubation presented in Figure 10 indicates that the Diazinon 60 EC treated soil obtained the greatest amount of Formazan (0.075 µmole/mL) followed by Marshal 20 EC (0.050 µmole/mL) which were significantly different from other insecticides treated soils. The lowest amount of Formazan was obtained from the control soil (0.046 µmole/mL). Dursban 20 EC and Admire 200 SL treated soils released same amount of formazan which was significantly higher from the control soil (Appendix 2).

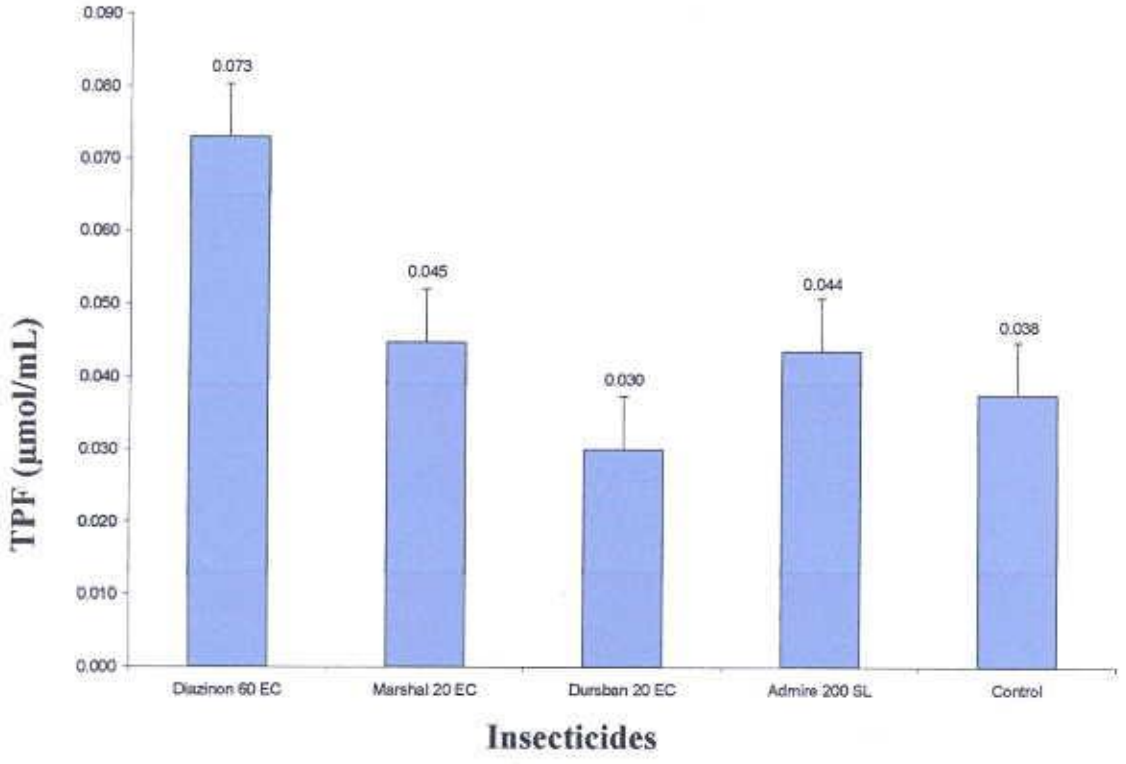

Figure 9. Comparative effects of 4 selected insecticides on dehydrogenase activity after 2 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

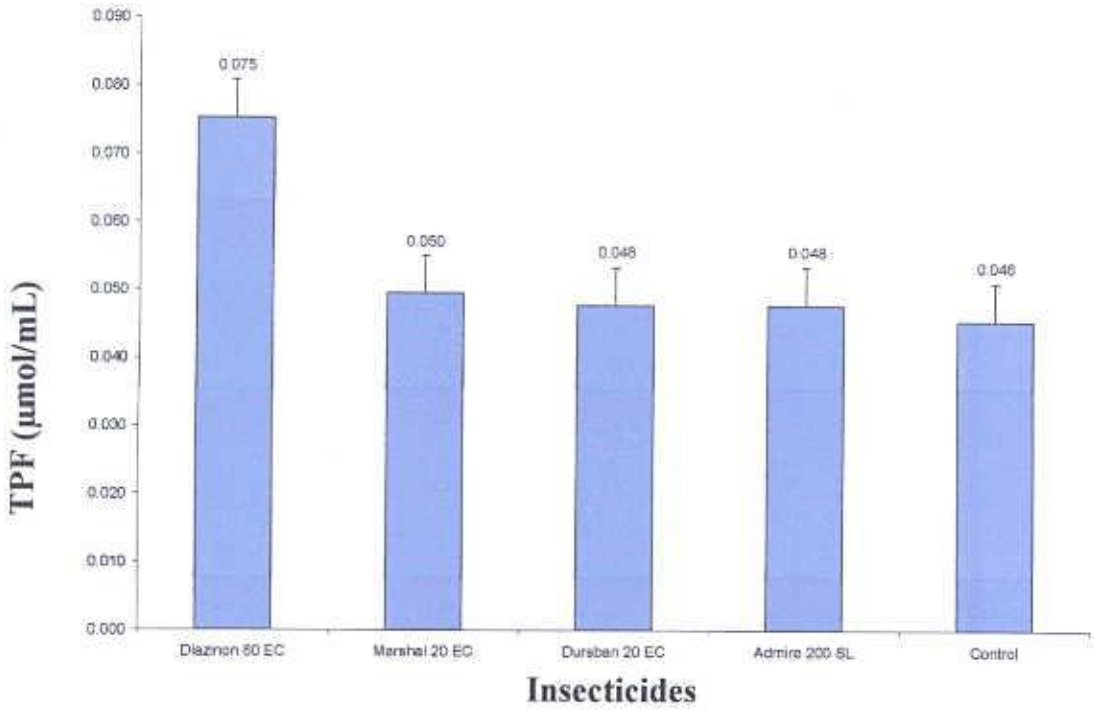

Figure 10. Comparative effects of 4 selected insecticides on dehydrogenase activity after 4 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

4.2.3 Incubation period: 8 days

After 8 days of incubation the highest amount of Formazan (0.079 µmole/mL) was released from Diazinon 60 EC treated soil, which was much higher than all other insecticides treated soil (Figure 11). The lowest amount of Formazan (0.037 µmole/mL) was release from Dursban 20 EC treated soil which was significantly lower than the control soil (0.059 µmole/mL). Statistically similar amount of formazan obtained from Marshal 20 EC and Admire 200 SL treated soils which were also significantly lower from control (Appendix 2).

4.2.4 Incubation period: 16 days

The highest amount of Formazan was obtained from Diazinon 60 EC (0.072 µmole/mL) treated soil after 16 days of incubation, which was much higher than any other insecticides treated soils and the lowest amount of Formazan was released from Dursban 20 EC (0.034 µmole/mL) as shown in Figure 12. Marshal 20 EC and Admire 200 SL treated soil released 0.060 µmole/mL and 0.063 µmole/mL formazan respectively, which was significantly greater than control soil (0.045 µmole/mL) (Appendix 2).

4.2.5 Incubation period: 24 days

The results presented in the figure 13 shows that after 24 days of incubation formazan formation was highest in Diazinon 60 EC treated soil (0.067 µmole/mL). Marshal 20 EC and Admire 200 SL treated soil released 0.049 µmole/mL and 0.056 mg/L formazan respectively, which was significantly greater than control soil (0.044 µmole/mL) (Appendix 2). The lowest amount (0.031 µmole/mL) of Formazan was released from Dursban 20 EC treated soil. (Appendix 2).

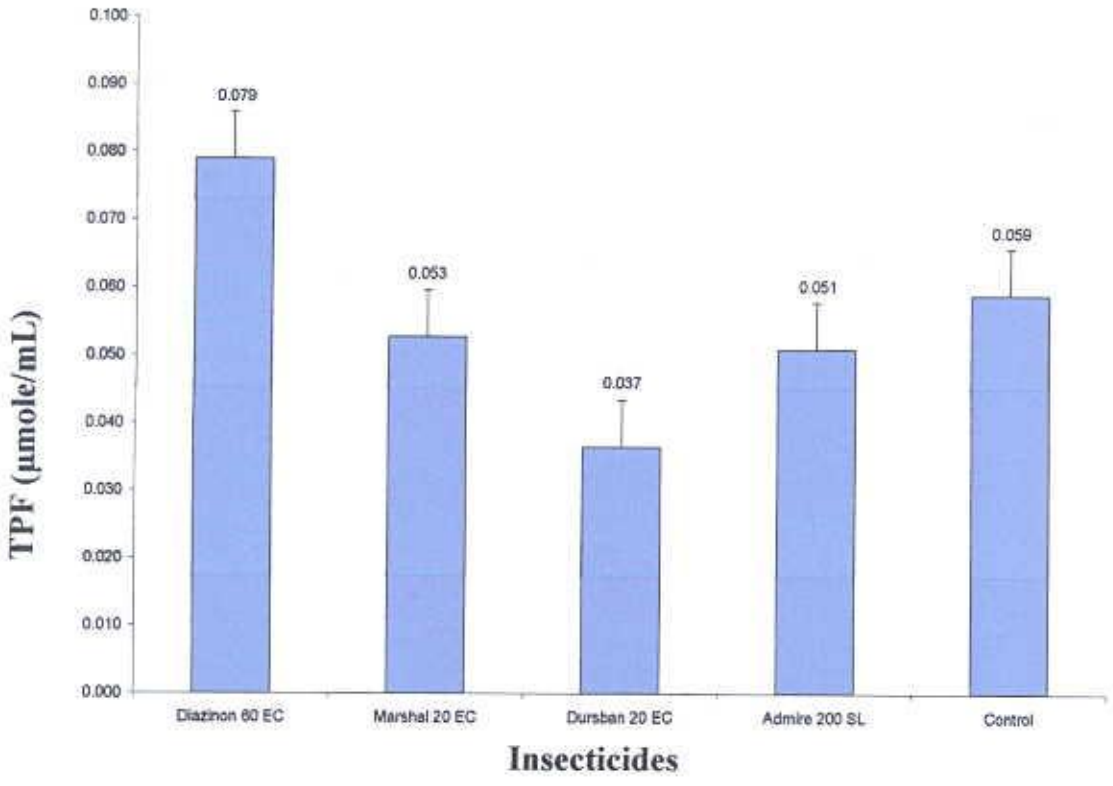

Figure 11. Comparative effects of 4 selected insecticides on dehydrogenase activity after 8 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

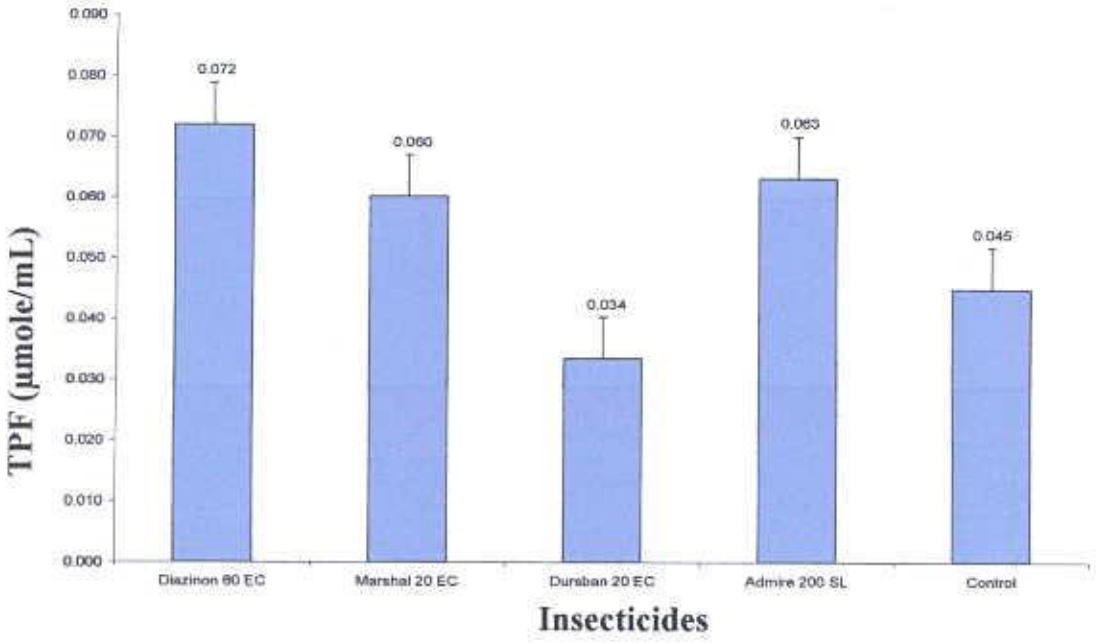

Figure 12. Comparative effects of 4 selected insecticides on dehydrogenase activity after 16 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

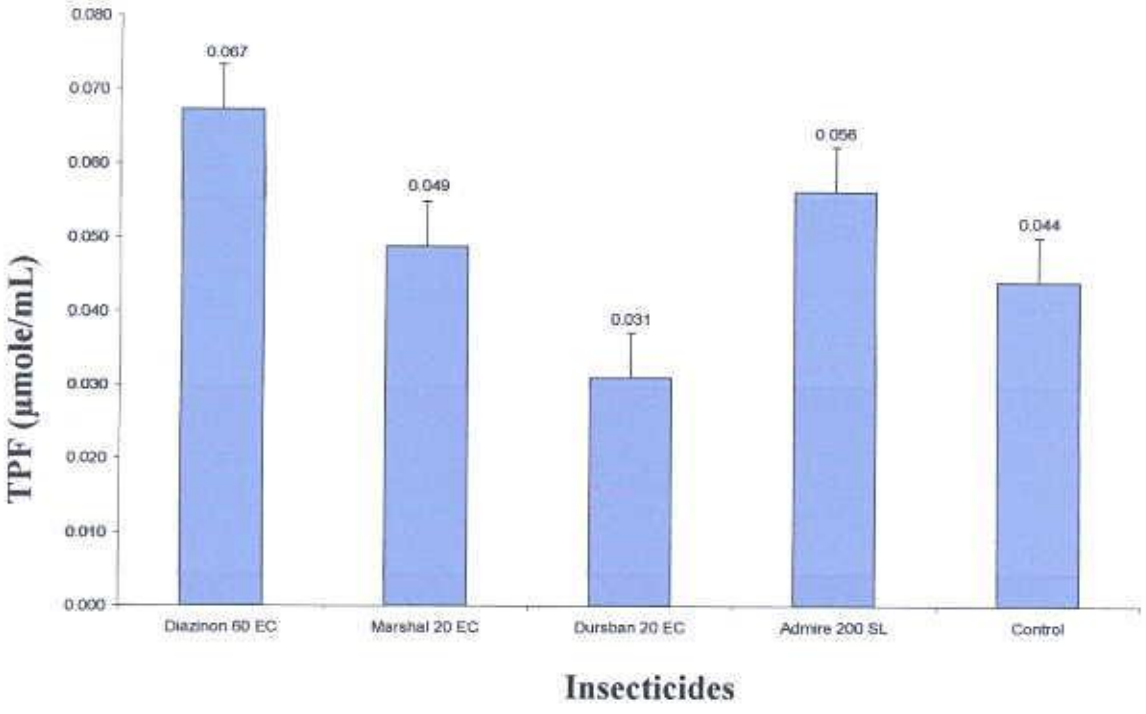

Figure 13. Comparative effects of 4 selected insecticides on dehydrogenase activity after 24 days of insecticides application in the laboratory. (Values are significantly different ($p<0.01$) in DMRT (Duncan Multiple Range Test). The T shaped beams represent the standard errors).

The experiment thus revealed that all the insecticides tested in the present study were useful in terms of dehydrogenase activity i.e. microbial population. Diazinon 60 EC had consistently high positive dehydrogenase effect from starting incubation, continuing even up to 24 days of incubation. Similar but slightly less positive effect than that of Diazinon 60 EC was observed in case of Admire 200 SL, Marshal 20 EC and Dursban 20 EC.

In the investigation of dehydrogenase activity, the insecticides treated soils gave high formazan yields than that of the controlled soil. The highest formazan production was obtained, in Diazinon 60 EC treated soil. All of the studies were done at room temperature for the dehydrogenase activity determinations.

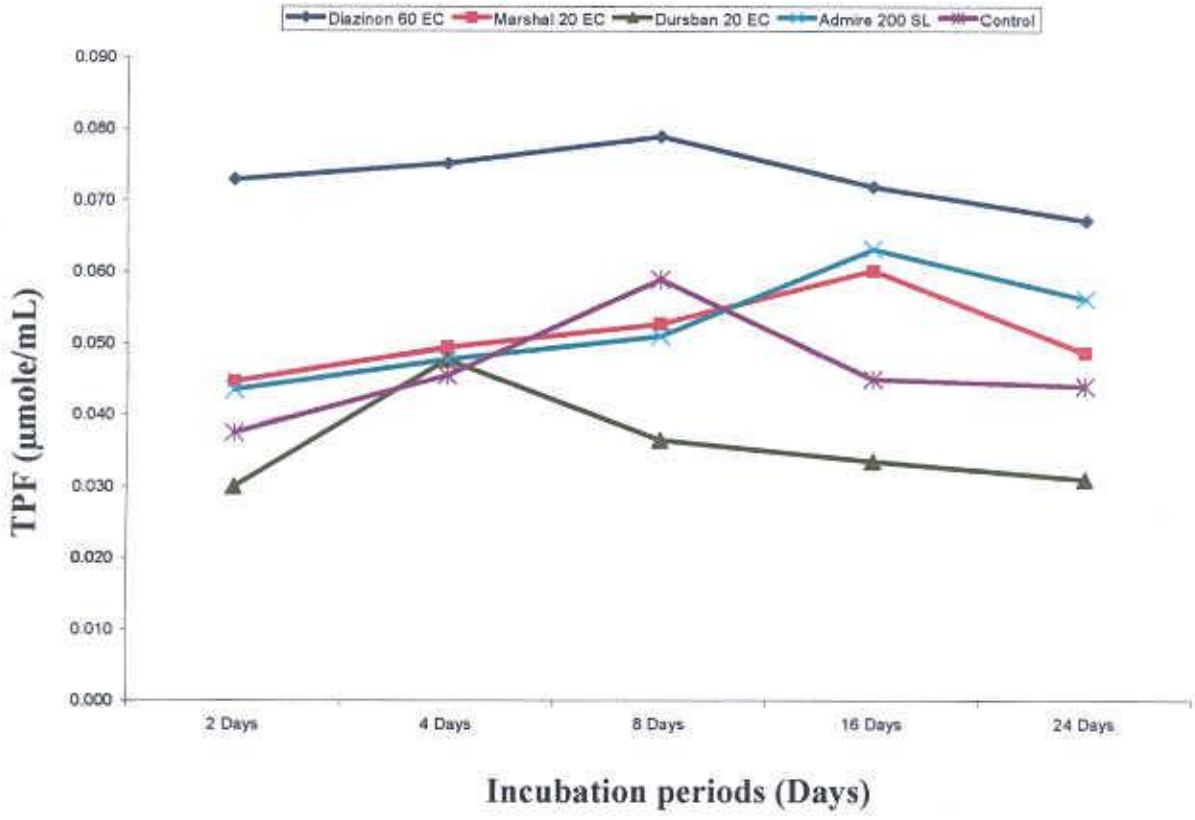

Figure 14. Trend of dehydrogenase activity from 4 selected insecticides treated soils and untreated soil (control) at different time intervals of incubation in the laboratory.

Temperatures lower than 37°C 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 (37^0C) but the reaction rates were too slow as formazan production was lower. A phenomenon somewhat similar to the later was studied by Stevenson (1956), who pointed out that nutrients become more readily available when dry soil is remoistened. In addition, however, there is probably some breaking of dormancy for the cells and depending on the duration of incubation of the 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 whether a soil has been preincubated, the dehydrogenase curves (formazan production) do not remain linear. It is possible, therefore, that accumulations of metabolically generated CO_2 in soil might slow the metabolic rates of the resident microorganisms. In this regard, it is of interest that dehydrogenase activity has been reported to be closely correlated with CO_2 release from soil (Skujins, 1973).

The foregoing discussion pinpoints some of the problems that affect interpretations of dehydrogenase determinations that use longer incubations. Ross (1971) studied soil dehydrogenase determinations conducted at 30°C. He too concluded that 6-h incubation was more valid than longer incubations; however, he recommended a 1-h anaerobic incubation. It should be pointed out that, even for 6-h determinations, speed is required in making the additions to the soil and mixing it so that all samples will receive equal incubation time. This is particularly true when large numbers of samples are being evaluated. A 1-h anaerobic incubation with its extra manipulations may prove difficult in this regard.

CHAPTER V

SUMMARY AND CONCLUSION

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An 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 to know the impact of 4 selected insecticides on soil microorganisms and the impact was expressed as the CO_2–C evolution and dehydrogenase activity (TPF formed) per gram of soil. The soil samples were incubated and the data were taken after 2, 4, 8, 16 and 24 days of incubation for the determination of the microbial activity.

The results can be summarized as; after 2 days of incubation the highest amount of CO_2 (74.81 µg CO_2 /g soil) was recorded from Marshal 20 EC treated soil. The lowest amount of CO_2 (37.57 µg CO_2 /g soil) was recorded from the untreated soil (Control). After 4 days of incubation the soil sample treated with Admire 200 SL released the highest (126.5 µg CO_2 /g soil) and Diazinon 60 EC treated soil released the lowest amount of CO_2 (60.17 µg CO_2/g soil). After 8 days of incubation the highest amount of CO_2 (281.2 µg CO_2/g soil) was released from Marshal 20 EC treated soil while the lowest amount of CO_2 (104.2 µg CO_2/g soil) release was observed in control soil. After 16 days of incubation, Admire 200 SL treated soil evolved the highest amount of CO_2 (222.4 µg CO_2/g soil) but the control soils released lowest amount of CO_2 (141.6 µg CO_2/g soil). After 24 days of incubation CO_2 evolution was highest in Marshal 20 EC treated soil (142.4 µg CO_2/g soil) while the lowest amount (59.41 µg CO_2/g soil) of CO_2 was released from untreated soil.

The result showed that the amount of CO_2 evolution is higher in all insecticides treated soil than the control soil after 2 days of incubation, which indicates the stimulatory effect of each insecticide on soil microorganisms during this period. Moreover, the greater amount of CO_2 evolution in Marshal 20 EC treated soils

indicates the greater activity of soil microorganisms. Marshal 20 EC had shown consistently higher positive stimulatory effect starting right from its application and continuing even up to 8 days of incubation where it was highest than other treated soil and also other incubation. On the other hand Diazinon 60 EC and Dursban 20 EC showed lower effect on microbial activity at 4 days of incubation with compared to control but the microbial activity increased there after (figure 8).

Dehydrogenase values were determined for tested soils after they had incubated for 2, 4, 8, 16 and 24 days with TTC. These soils produced more formazan than the soil not receiving any insecticide. Dehydrogenase values at different time intervals of incubation were shown in Figure 9-13 and Appendix 2.

The formation of formazan was highest in Diazinon 60 EC treated soil at all the incubation i.e. 2, 4, 8, 16 and 24 days of incubation. The lowest amount of formazan was obtained in Darsban 20 EC treated soil at all the incubation periods except at 4 days of incubation (figure 14). It was also lower than the untreated soil (control). The formation of formazan in Marshal 20 EC and Admire 200 SL treated soils were higher than control soil except at 8 days of incubation.

The experiment thus revealed that all the insecticides tested in the present study were useful in terms of dehydrogenase activity i.e. TPF formation. Diazinon 60 EC having consistently higher positive dehydroenase effect starting right from its application and continuing even up to 24 days of incubation. Similar but slightly less positive effect than Diazinon 60 EC was observed in case of Admire 200 SL, and Marshal 20 EC. The insecticides tested in soil for the dehydrogenase activity determination gave high formazan yields than that of the controlled soil except Dursban 20 EC treated soil. Good formnazan production was obtained with Diazinon 60 EC. All of the studies used a room incubation temperature during the dehydrogenase determinations.

Therefore, it can be concluded that all the 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 control treatment. In this experiment only the duration of incubation is considered. As our country is located in the semi arid region the temperature varies in summer and winter drastically and soil become saturated in the monsoon. As a result the pH of the soil changes. 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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APPENDICES

APPENDICES

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

Treatments	CO_2 evolution (µg /g soil)				
	2 Days	4 Days	8 Days	16 Days	24 Days
Diazinon 60 EC @ 3.0 ml/L	43.88d	60.17e	118.6d	209.3b	103.98c
Marshal 20 EC @ 2.0 ml/L	74.81a	110.9b	281.2a	171.9c	142.4a
Dursban 20 EC @ 2.0 ml/L	52.05c	75.66d	126.7c	163.7d	89.32d
Admire 200 SL @ 4.0 ml/L	65.89b	126.5a	162.1b	222.4a	133.4b
Control	37.57e	88.97c	104.2e	141.6e	59.41e
LSD (0.01)	1.950	1.959	2.597	2.874	1.784
CV%	1.65	0.98	0.76	0.73	0.78

Appendix 2. Effect of 4 selected insecticides on TPF obtained (µmole/mL) after different periods of incubation in the Laboratory

Treatments	TPF obtained (µmole/mL)				
	2 Days	4 Days	8 Days	16 Days	24 Days
Diazinon 60 EC @ 3.0 ml/l	0.073a	0.075a	0.079a	0.072a	0.067a
Marshal 20 EC @ 2.0 ml/l	0.045b	0.050b	0.053c	0.060c	0.049c
Dursban 20 EC @ 2.0 ml/l	0.030d	0.048c	0.037d	0.034e	0.031e
Admire 200 SL @ 4.0 ml/l	0.044b	0.048c	0.051c	0.063b	0.056b
Control	0.038c	0.046d	0.059b	0.045d	0.044d
LSD (0.01)	0.018	0.002	0.014	0.004	0.003
CV%	4.89	3.84	2.60	2.74	2.96