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RESEARCH ARTICLE

EFFECT OF INSECTICIDES ON SOIL MICROBIAL RESPIRATION.

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Abstract

Laboratory experiment was conducted to study the effect of chlorpyrifos and cypermethrin insecticides on soil microbial respiration. In the experiment replicated trials were conducted on experimental field soil before application of selected insecticides using chlorpyrifos 20EC and cypermethrin 25EC at 0, 5, 10, 15, 20, 50 and 100ppm levels of fortification. The heterotrophic activities of soil micro-organisms were measured in terms of CO_2 evolved after incubation period spanning from 5, 20, 35 and 50 days. The results obtained in the experiment revealed that both chlorpyrifos and cypermethrin showed gradual decrease in CO_2 evolution for all levels of fortification as compare to untreated soil. But there was no adverse effect on soil microbial activity.

Introduction:-

Pesticides are toxic agrochemicals used frequently in the field to increase crop yields by controlling insect pest infestation. Thus pesticides have become an integral part of modern agriculture. Modern agriculture worldwide uses a variety of pesticides including insecticides, nematicides, herbicides and fungicides to optimize crop production (Lo ´ pez *et al.*, 2002; Cycon *et al.*, 2006). Pesticides when applied on crop as foliar spray, they fall on soil and affect the population and activity of beneficial microorganisms in soil as well as physico- chemical properties of soil. (Pandey & Singh, 2004) When pesticides are used in crop protection, the possibilities of these chemicals may exert certain effect on non target organisms along with targeted organisms. Microorganisms make up less than 1% of total soil volume (Purohit, 2003) and they are categorized into bacteria, actinomycetes, fungi, algae and protozoa (Rao, 1995). One of the main activities of these soil microorganisms is the decomposition of organic matter in the soil. 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). Therefore, soil respiration is a good index to evaluate the activity of microorganisms involved in organic matter decomposition. (Komal *et al.*, 1999) To evaluate toxic effect exerted by insecticides on soil microorganisms, soil respiration index is frequently used. (W. J. Jones & N.D. Ananyeva, 2001 & A.Kalia & S.K. Gosal, 2011)

The impact of insecticides on various soil parameters, including soil microbial respiration has been studied by many workers. (D.Sengupta *et al*., 2009, A.C. Das *et al*., 1995, Digrak M. and F. Kazanici, 2001) There are many results regarding favorable as well as adverse effects of insecticides on growth and activities of microorganisms in the soil. (S.Bhuyan *et al.*, 1992, Bujin X.U. and Z. Yongxi, 2000) but these effects on soil microbial activities are temporary. Also, the effects of different insecticides on the growth and activities of micro-organisms in the soil show variations as different groups of insecticides exhibit variations in toxicity. (Das A. C. and D. Mukherjee, 2000) In the present

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study investigations on effect of selected insecticides, namely cypermethrin and chlorpyrifos under controlled laboratory conditions on soil from the agricultural experimental farm in Nagpur, India has been undertaken.

Materials and Method:-

Materials:-

Soil from experimental field, cypermethrin 25EC, chlorpyrifos 20EC, barium chloride (BaCl2), NaOH, HCl, phenolphthalein indicator, conical flasks (500ml), glass vials (25ml), rubber corks with hook, burette, conical flask(250ml).

Method:-

Preparation of incubation chamber:-

Incubation chamber was prepared using 500ml. conical flask which was tightly fitted with rubber cork. The rubber cork was provided with a hook in order to suspend 25 ml glass tube inside the conical flask with the help of thread during experimentation.

Soil treatment with pesticides cypermethrin and chlorpyrifos:-

In incubation chamber 50gm soil was taken which was mixed with appropriate quantity of water in order to maintain field condition. Soil was then mixed with 10ml of cypermethrin insecticide suspensions prepared in water of different concentration i.e. 0ppm, 5ppm, 10ppm, 15ppm, 20ppm, 50ppm and 100ppm.Then glass tube (25ml) filled with 10ml of 0.5N NaOH was kept suspended in each incubation chamber with the help of thread hooked from the cork inside the chamber. Rubber stopper was fitted tightly.

Similarly incubation chamber with 50 gm soil were taken which were mixed with appropriate quantity of water in order to maintain field condition and then was mixed with 10ml of chlorpyrifos insecticide suspensions, prepared in water of different concentration i.e. 0ppm, 5ppm, 10ppm, 15ppm, 20ppm, 50ppm and 100ppm.Then glass tube (25ml) filled with 10ml of 0.5N NaOH was kept suspended in each incubation chamber with the help of thread hooked from the cork inside the chamber. Rubber stopper was fitted tightly.

Incubation of soil with pesticides cypermethrin and chlorpyrifos:-

Effect of cypermethrin and chlorpyrifos insecticides on soil micro flora was evaluated by fortification of soil at 0,5,10,15,20,50 and 100ppm using three replicated sets. Observations were recorded at an interval of 5days, 20 days, 35days and 50 days in terms of CO_2 evolved in the incubation chamber.

Method of recording observations:-

The glass tube in the chamber was taken out at 5, 20, 35 and 50 days from the start of experiment. Every time content of the glass tube was used for titration and the glass tube was replaced immediately with fresh vial containing the 10 ml of 0.5N NaOH. The content of the vial was titrated with 0.7N HCl using 10ml of 1N $BaCl_2$ and phenolphthalein indicator.

The amount of CO_2 evolved from the soil was calculated by formula given below:

$$CO_2 \text{ evolved (mg/g soil)} = \frac{(B-A)\times 2.2}{0.1\times N/50}$$

Where,

A= ml of 0.7NHCl required to neutralize NaOH in the vial from incubation chamber.

B= ml of 0.7NHCl required for blank reading.

N= Normality of acid used (1ml of 0.1NHCl is equivalent to 2.2mg CO_2)]

The calculated quantity of CO_2 per gram soil from four observations for the incubation period of 5days, 20 days, 35days and 50 days were considered together and used for comparison. Following photographs show assembly prepared and used during recording observations.

Result:-

Carbon dioxide liberated from the soil in incubation chamber was trapped in NaOH solution and by titration with standard acid (HCl); the quantity of CO_2 was calculated in mg/g soil for the total incubation period of 50 days. The soil for the experiment was collected from experimental farm and was fortified with cypermethrin and chlorpyrifos at the level of 0,5,10,15,20,50 and 100 ppm using the calculated quantity of formulated emulsifiable concentrate. Initially the CO_2 was measured after 5days of fortification and subsequently it was measured at an interval of 15 days (readings correspond to 20days of incubation period), 30days (readings correspond to 35days of incubation period) and 45 days (readings correspond to 50days of incubation period). The total quantity of carbon dioxide liberated in all four observations was taken for comparison.

Effect of pesticides, cypermethrin on soil microbial respiration:-

The quantity of CO_2 liberated (mg/g soil) from incubated soil with cypermethrin in incubation chamber at various levels of fortification and at different incubation period is presented in Table 1.

Table 1:- Influence of Cypermethrin (25EC) on CO_2 evolution from soil (incubation period from 5days to 50days)

Influence of Cypermethrin 25EC on soil respiration				
Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	5 Days	20 Days	35Days	50Days
0	3.63(±0.16)	2.6(±0.0836)	1.86(±0.1645)	0.96(±0.1474)
5	3.5(±0.08)	2.5(±0.0816)	1.8 (±0.0816)	0.95(±0.0408)
10	3.5(±0.08)	2.47(±0.0471)	1.76(±0.3522)	0.93(±0.0918)
15	3.4(±0.00)	2.45(±0.08167)	1.75(±0.040)	0.86(±0.2318)
20	3.4(±0.086)	2.42(±0.02449)	1.73(±0.1173)	0.86(±0.1428)
50	3.36(±0.216)	2.38(±0.1405)	1.6(±0.405)	0.73(±0.0936)
100	3.3(±0.816)	2.33(±0.133)	1.0(±0.416)	0.56(±0.098)

In untreated soil (0ppm level of fortification) the CO_2 evolved showed gradual decreasing trend from 5 days to 50 days of incubation period. The amount of CO_2 evolved was 3.63mg/g, 2.6mg/g, 1.0mg/g and 0.56mg/g for 5, 20, 35 and 50days respectively.

Whereas in case of soil with 5, 10, 15 and 20ppm fortification level of cypermethrin, the amount of CO_2 evolved also showed gradual decrease during 5days to 50 days which was in the range of 3.5 to 3.4.mg/g to 0.95to0.86mg/g. which when compared with untreated soil was at par.

However, relatively more reduction in CO_2 evolved was noticed as compare to untreated soil (0ppm level of fortification) at 50 and 100 ppm fortification level of cypermethrin during 5days to 20days and CO_2 quantity recorded was 3.36 to3.3mg/g and 2.38 to 2.33 mg/g soil, respectively. Also, after 35 days of incubation period, the same trend was found i.e. as compare to untreated soil the CO_2 evolved showed gradual decrease with increasing level of fortification. For 35 days of incubation period quantity of CO_2 recorded for untreated soil was 1.86 mg/g whereas, for increasing levels of fortification from 5 to 100ppm, it was recorded as 1.8, 1.76, 1.75, 1.73, 1.6 and 1.0 mg/g, respectively. This decreasing trend in CO_2 evolution was observed till 50 days of incubation period. The quantity of CO_2 evolved for untreated soil after 50 days was 0.96 mg/g and for soil incubated with increasing levels of fortification from 5 to 100ppm, it was 0.95, 0.93, 0.86, 0.86, 0.73and 0.56 mg/g, respectively.

Effect of chlorpyrifos on soil microbial respiration:-

In the laboratory experiment performed on farm soil, the quantity of CO_2 liberated (mg/g soil) from incubated soil with chlorpyrifos in incubation chamber at various levels of fortification and at different incubation period is presented in Table 2.

Table 2:- Influence of Chlorpyrifos (20EC) on CO_2 (For incubation period from 5days to 50days).

Influence of Chlorpyrifos 20EC on soil respiration				
Level of fortification in (ppm)	CO_2 evolved in mg/g soil			
	5 Days	20 Days	35Days	50Days
0	3.65(±0.0816)	3.51(±0.2174)	3.28(±0.01414)	3.19(±0.1632)
5	3.58(±0.1582)	3.48(±0.01414)	3.25(±0.01632)	3.15(±0.0816)
10	3.46(±0.0816)	3.42(±0.0216)	3.21(±0.08164)	3.10(±0.0216)
15	3.41(±0.1517)	3.38(±0.0294)	$3.19^{\pm 0.0216}$)	3.07(±0.01414)
20	3.35(±0.0163)	3.21(±0.0496)	3.08(±0.0216)	2.92(±0.0816)
50	3.26(±0.0141)	3.15(±0.01414)	2.87(±0.01632)	2.75(±0.0294)
100	3.18(±0.029)	3.06(±0.1267)	2.55(±0.0216)	2.48(±0.222)

The CO_2 evolved from untreated soil (0ppm) for incubation period of 5 days was 3.65 mg/g which was slowly decreased after incubation period of 20 days to 3.51mg/kg and then showed further decrease to 3.28 and 3.19 mg/g for incubation period of 35days and 50 days, respectively.

Similar trend was observed in case of soil treated with chlorpyrifos with fortification level 5, 10, 15, 20, 50 and 100ppm for 5 to 50 days of incubation period. With increasing levels of fortification, the quantity of CO_2 evolved was gradually decreased in the range of 3.58 to3.18 mg/g for 5 days of incubation period. whereas for incubation period of 20 days it decreased gradually from 3.48 mg/g to 3.06mg/g. and for 35 and 50 days of incubation period the quantity of CO_2 evolved were 3.28 to 2.55mg/g and 3.19 to 2.48 mg/g,respectively which shows with increasing level of fortification there was gradual decrease in CO2 evolved.

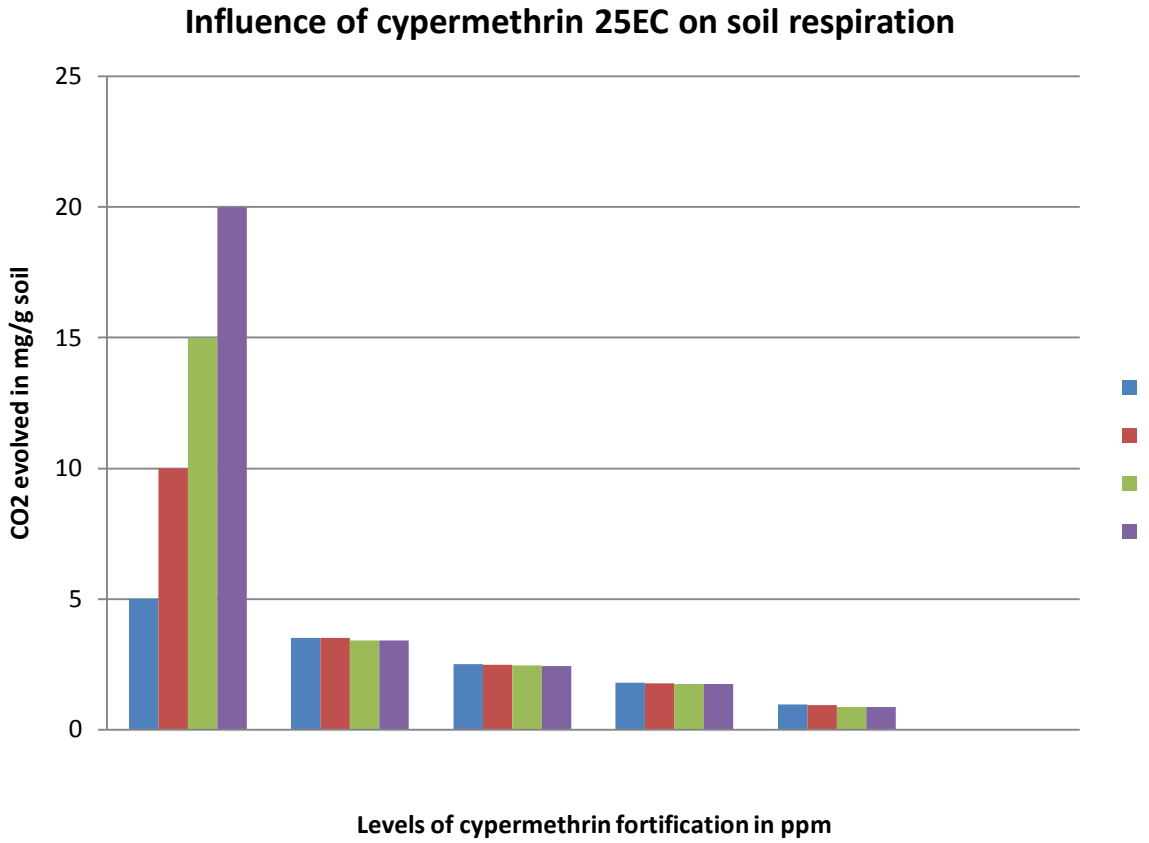
The quantity of CO_2 evolved from untreated soil (0ppm) for incubation period of 5 to 50 days when compared with soil fortified with 5 to 100ppm chlorpyrifos, it was observed that there was continuous decrease in CO_2 evolved in case of treated soil for 5 to 50 days and fortified with 5 to 100ppm chlorpyrifos.

However, relatively more reduction in CO_2 evolved was noticed as compare to untreated soil (0ppm level of fortification) at 50 and 100 ppm fortification level of chlorpyrifos during 5days to 50days and CO_2 quantity recorded was 3.26 to3.18 mg/g, 3.15 to 3.06 mg/g, 2.87 to 2.55 mg/g and 2.75 to 2.48 mg/g soil, respectively.

Discussion:-

When soil was treated with different fortified levels of cypermethrin, the CO_2 that evolved in the incubation chamber from 5days to 50 days indicated that there was a gradual decrease in microbial activity when compared from 5days to 50days with increasing fortification levels as seen in Table 1. However, when compared with untreated soil, the treated soil, irrespective of the concentrations of the cypermethrin mixed, showed minimal toxic or adverse effect on the soil micro-flora as seen in Fig.1. Thus, our result supported the work of M.A.Latif *et al.*, 2008 who reported that cypermethrin had no adverse effect on soil microbes.

Fig 1:- Graph showing influence of Cypermethrin (25EC) on CO_2 evolved (For incubation period from 5days to 50days).



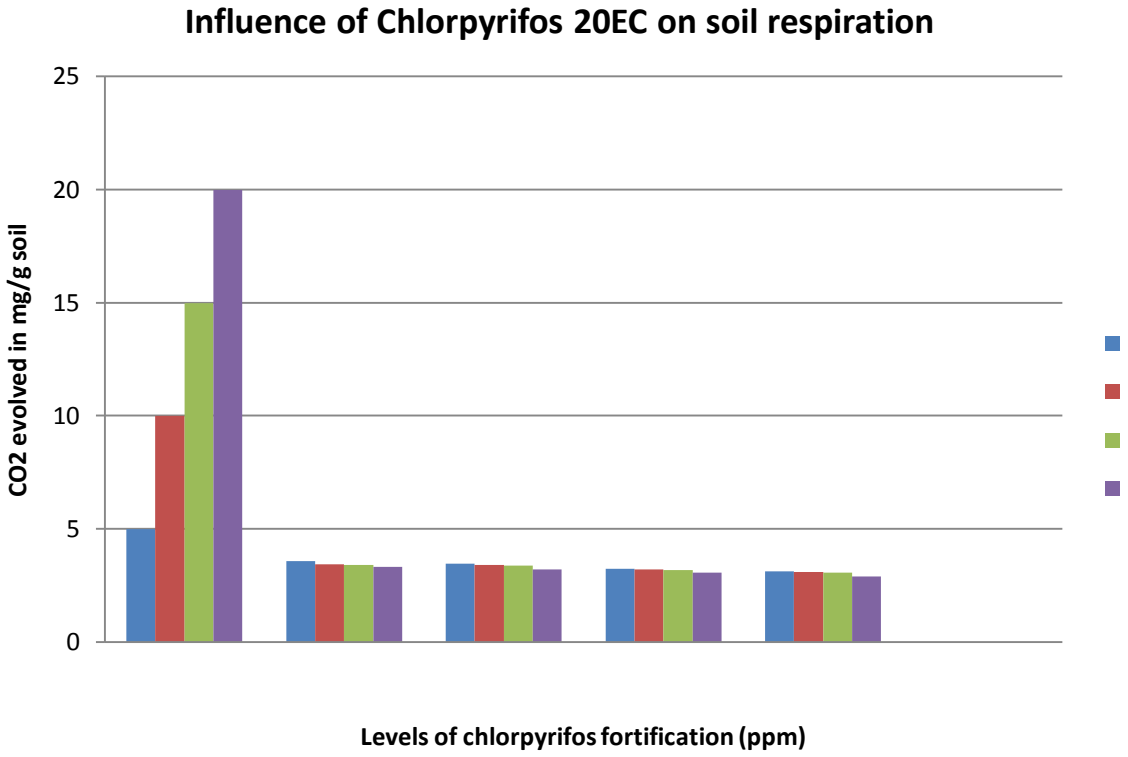
Similar findings were reported by Sonia Sethi and Saksham Gupta, 2013 in their comparative studies on, impact of pesticides on soil microbial biomass carbon assessed the effect of five pesticides namely cypermethrin, malathion, imidacloprid (victor), monocrotophos (monocil) and dimethoate (tafgor) and five biopesticides on soil microbial biomass carbon under laboratory condition. All pesticides showed short lived transient toxic effect on soil microbial biomass carbon.

Moumit Roy Goswami *et al.*, 2012 reported that application of cypermethrin insecticide on soil at their recommended dose showed short term weak transient toxic effect on soil biomass and respiration.

When soil was treated with different fortified levels of chlorpyrifos, the CO_2 that evolved in the incubation chamber from 5 days to 50 days showed decrease in the CO_2 evolution. This is evident in Table 2 and these results were in agreement with the results reported by Pandey and Singh (2004).

Ahmed, S. and Ahmed, M. S., 2006 and Nazia Sultan *et al*., 2010, reported in their study that chlorpyrifos insecticide treatments displayed a short-term inhibitory effect on microbial activity which were in agreement with the results obtained in present study and as seen in Fig.2.

Fig 2:- Graph showing influence of Chlorpyrifos (20EC) on CO_2 evolved (For incubation period from 5days to 50days)



Soil microbial activity in terms of CO_2 evolved was decreased from 5 to 50 days (Table2) with slight adverse effects on microbial activity attributed by chlorpyrifos present in the soil. These observations were in agreement with the observations reported by M.A.Latif *et al.*, 2008 who reported that chlorpyrifos had inhibitory effect on microbial respiration when observed after 24 or 32 days of incubation.

Masum Billah *et al.*, 2014, conducted an experiment in the laboratory 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, reported that chlorpyrifos (Dursban) 20 EC had adverse effect on microbial activity at initial period of incubation and also reported in their studies that as compared to control there was gradual decrease in CO_2 evolved for initial period of incubation. These results are in agreement with results obtained in the present findings.

S.S. Sarnaik *et al.*, 2004, in their studies on effect of application of different pesticides to soysbean on the soil microflora, reported that chlorpyrifos had no adverse effect on soil microflora which was in agreement with the present study.

Conclusion:-

The results obtained in the present study reveal that cypermethrin 25EcC and chlorpyrifos 20EC applied on experimental farm soil had minimal inhibitory effect as compare to untreated soil for different levels of fortification of insecticides applied as well as for different days of incubation period ,on soil microbial activity.

Therefore, it may be concluded that cypermethrin and chlorpyrifos can be safely used in crop protection using appropriate concentrations so that their effects will not be harmful to soil microorganisms.

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