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Effect of Pesticides on the Soil Microbial Activity

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Abstract. -The purpose of this study was to evaluate the effect of commonly used pesticides such as Pendimethaline, Trifluralin, Glyphosphate, 2, 4-D, and MCPA (Chwastox) on microbial activities in soil. Two types of clean soils were amended with recommended level of pesticides and incubated in the laboratory at 35°C for 15 days. Microbial activities in the form of CO_2 production were measured during incubation at 1, 2, 3, 4, 5, 7, 9, 11, and 15 day intervals. CO_2 production was not affected substantially by any of the applied doses of pesticides. However, the total amount of CO_2 produced during 15 days was suppressed by all pesticides, except MCPA (Chwastox). The effect of pesticides on microbial activities varied greatly with the type of pesticides used. The MCPA Chwastox did not exert any inhibitory effect on the respiratory rate of microbes, while other selected pesticides showed highly toxic effect on soil microbial activity.

Keywords: Pendimethaline, Trifluralin, Glyphosphate, 2, 4- D, MCPA (Chwastox), soil microbes

INTRODUCTION

Insecticides generally are the most hazardous to the environment, followed by fungicides and herbicides. This is a generalized statement, because certain herbicides are highly toxic and present a greater hazard to the environment than some insecticides. Thus, one has to be specific about which pesticide (including its dosages and methods of application) is being investigated in an ecological study. An estimated quantity of about 2.5 million tons of pesticides about US$ 16.3 billion is applied in world agriculture costing annually (Helsel, 1987). Despite the use of this huge amount of pesticides, besides several other controls methods, pests manage to destroy in the world 36% of all potential crops before harvest. There is now overwhelming evidence that some of these chemicals pose risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993). No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden are shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world-wide deaths and chronic diseases due to pesticide poisoning number about 1million per year (EF, 1999).

The increased use of pesticides in agricultural soils causes the contamination of the soil with toxic chemicals (Muñoz-Leoz *et al.*, 2013). When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Zhao *et al.*, 2013). The microbes play an important role in the soil ecosystem (Khan *et al.*, 2010), and their functions (Khan *et al.*, 2007) are very crucial in nutrient cycling and decomposition (Lorenzo *et al.*, 2001).

The study of pesticide effects on non-target populations is an accepted strategy to evaluate its associated potential environmental risks. Among non-target populations, soil microorganisms are extremely important, since they play an essential role in nutrient turnover (Aneja, 2004), maintaining generative capacity in agro-ecosystems (Bohlen, 2002). The processes of ecological succession are, among other factors, mediated by microorganisms and depend on a fine balance of their population dynamics (Kennedy, 1999). Under these circumstances, the impact inflicted on soil microbial populations caused by a specific pesticide is a potential indicator of the toxicity level of this product, and may represent a component of a broad study aiming to evaluate its potential impact on the environment (Kent, 2002).

Absorption of pesticides into the soil and their persistence varies with the composition, pH,

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and temperature of the soil. Most organophosphate hydrocarbon can be degraded by plants, but organo-chlorinated pesticides cannot, so they are taken up by them when present in soil (Gao *et al.*, 2013; Gul and Khan, 2001). This study was aimed at evaluating the commonly used pesticides for their adverse effects on soil microbial activity.

MATERIALS AND METHODS

Soil samples and preparation

Laboratory incubation experiment was conducted to assess the effect of five different pesticides on microbial activity (respiration rate) in two different soils. Soil samples were collected from two sites (Pirsabak and KattiKhel) located near Charsadda on Charsadda-Nowshehra Road.

Soil of Pirsabak site was silty clay loam, calcareous, non-saline upto more than 3ft deep and was classified as coarse loamy mixed hyperthemic-typicustocript. The sample was taken from the depth of 90 cm. Its pH was 9.3, had 0.81% organic matter and 0.47% organic carbon. Soil of KattiKhel site possesses the same characteristic as Pirsabak soil except that this soil was saline and was classified as coarse loamy mixed hyperthemi-cacrichaplaquepts. The soil samples were taken from the depth of 90 cm. Its pH was 9.9, had 1.26% of organic matter and 0.73% of organic carbon.

After collection, soil samples were broken down gently by hand. Stones and visible plant roots liters were removed and sieved (2 mm). Field moist soil samples were used in the incubation. Triplicate soil samples from each soil series were analyzed for key soil properties.

Treatment of soil samples with pesticides

Following pesticides, fungicides and insecticides were used in this study.Pendimethalin$^{33\ EC}$ active ingredients (a.i.) 33% W/V Trifluralin$^{48\ EC}$ *a.i.* 46.3% the herbicide Glyphosate ^{41EC}*i.v.* 41% W/W, and insecticide. MCPA ^{50EC}*a.i.* 44.25% of Chwastox; and 2, 4-Dichlorophenoxy Acetate (2, 4-D) *a.i.* were 92%**.**

Measurement of microbial activity in soil

Microbial activities were measured in the form of CO_2 evolved during incubation by the method of Andreson *et al.* (1980). In this method, 50 g soil often samples as taken in a 500 ml conical flask. The samples were amended with appropriate pesticides in solution form and thoroughly mixed. Four (2 for Pirsabak and 2 for KattiKhel) flasks were kept amended with pesticides to act as control treatment. Each treatment was arranged in duplicate, thus a total of 26 flasks were arranged.

A 10 ml of 0.3M NaOH solutions was taken in a glass vial and suspended carefully in each flask with the help of a string. The flasks were sealed properly with rubber bungs to avoid any gaseous exchange between the flasks and outside atmosphere. A blank, in duplicate, was also run to account for the amount of CO_2 already present in the flask's atmosphere. The flasks were placed in the incubator at 35ºC and taken out at 1, 2, 3,4,5,7,9,11 and 15 days of incubation. At termination of an experiment the vial was carefully taken out of flask and the NaOH solution was transferred to clean 250ml flask. For next incubation fresh NaOH solution was suspended in the same flask and returned to the incubator. The procedure was repeated at the end of each previous incubation period. After adding 10ml of 1M $BaCl_2$ solution and few drops of phenolphathalein, to the recovered NaOH solution, it was titrated against 0.1M HCl solutions until the pink color disappeared.

During the reaction one mole of CO_2 neutralizes two moles of NaOH. The amount of CO_2 produced was calibrated as g/g of moist soil/h.

RESULTS AND DISCUSSION

The effect of pesticides was measured on microbial activity in term of CO_2 production in the pesticides amended and un- amended soil samples. The results obtained are presented below.

Rate of CO_2 evolution

Rate of CO_2 evolution in the pesticides amended and un-amended (control) soil samples was measured from day 1 to day 15 of incubation period (Table I). The results showed that no considerable differences in the rate of CO_2 evolution were observed between the pesticides amended and control soils on day 1 to day 15 of incubation period.

Table I.- Rate of CO_2 evolution (mg CO_2 kg^{-1} soil hr^{-1}) in the pesticides amended and un-amended soil samples during different incubation periods.

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 9	Day 11	Day 15
Pirsabak Soil									
T1 (Control)	858	784	784	708	616	321	384	215	54
T2	858	773	756	642	641	328	211	113	47
T3	858	793	600	659	639	420	186	170	76
T4	858	825	702	681	642	349	222	207	158
T5	858	783	670	687	678	399	185	186	93
T6	858	609	601	669	658	427	215	143	115
KattiKhel soil									
T7	858	858	858	709	641	335	235	158	90
T8	858	858	858	713	682	181	191	148	102
T9	858	858	858	725	641	431	241	144	104
T10	858	858	858	743	698	327	256	129	97
T11	858	858	858	743	653	387	228	196	108
T12 (Control)	858	858	858	729	629	415	140	194	194

Pirsabak soil: T1, Soil only (control); T2, Soil+Trifluralin at 1000ml/ha; T3, Soil+Pendimethalin at 1000ml/ha; T4, Soil+MCPA (Chwastox) at 500ml/ha; T5, Soil+2,4-D at 750g/ha; T6, Soil+Glyphosate at 1900 ml/ha. **KattiKhel soil:** T7, Soil+Trifluralin at 1000ml/ha; T8, Soil+Pendimethalin at 1000ml/ha; T9, Soil+MCPA (Chwastox) at 500ml/ha; T10, Soil + 2,4-D at 750g/ha T11, Soil+Glyphosate at 1900 ml/ha; T12, Soil only (control).

On first day of incubation, there was no difference in CO_2 production between pesticide-amended and un-amended soils and the amount of CO_2 produced was 858mg CO_2 kg^{-1}soil hr^{-1}. Similarly, no difference was observed between soils of Pirsabak and KattiKhel series. On second day of incubation, the CO_2 production was slightly reduced from 609 to 825mg CO_2 kg^{-1}soil hr^{-1} in all treatments of Pirsabak soil compared to day 1 (Table I). In the KattiKhel series the CO_2 production remained the same on the second day of incubation in all pesticides amended and un-amended samples.

On third day of incubation, the overall production of CO_2 in Pirsabak soil was further reduced and ranged from 600 to 756mg CO_2 kg^{-1} soil hr^{-1} in the pesticide amended treatments compared to control (784mg CO_2 kg^{-1} soil hr^{-1}). Like the first two days, on third day of incubation the KattiKhel soil treatments were not shown any change in the amount of CO_2 production (858mg CO_2 kg^{-1} soil hr^{-1}) in both amended and un-amended soils. The pattern of CO_2 production in all the treatments on the fourth day of incubation was almost similar to that of the third day of incubation in the Pirsabak series. In case of KattiKhel soil, the CO_2 production in the pesticides amended treatments showed a decrease and ranged from 709 to 743mg CO_2 kg^{-1} soil hr^{-1} as compared 729mg CO_2 kg^{-1} soil hr^{-1} control soil. However, the CO_2 production was remained the same in both series of soils on the fifth day as observed on the fourth day of incubation. There were only minor differences in CO_2 production between similar treatments on both days of incubation.

On day 7 of incubation, the CO_2 production in Pirsabak soil was slightly increased and ranged from 328 to 427mg CO_2 kg^{-1} soil hr^{-1} in the pesticide amended soil as compared to 321 mg CO_2 kg^{-1} soil hr^{-1} in the unamended soil. Except for MCPA (Chwastox) amendment, the rate of CO_2 production was reduced from 181 to 431mg CO_2 kg^{-1} soil hr^{-1} compared to 415mg CO_2 kg^{-1} soil hr^{-1} in the control KattiKhel soil (Table I). On 9th and 11th day of incubation, the pattern of CO_2 production in all the treatments was similar to each other. The least reduction in the CO_2 production occurred in the pesticides amended soils of Pirsabak.

On 15th day of incubation, the overall production of CO_2 evolution in all the treatments was reduced. In the Pirsabak series, the rate of CO_2

Table II.- Cumulative CO_2 production (mg CO_2 kg^{-1} Soil) in the pesticides amended and un- amended soil samples during different incubation periods.

Treatments*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 9	Day 11	Day 15	Mean
Pirsabak Soil										
T1 (Control)	858	1642	2426	3134	3750	4071	4455	4070	4724	3237
T2	858	1631	2387	3029	3676	4004	4215	4328	4375	3167
T3	858	1651	2251	2910	3545	3969	4155	4325	4401	3118
T4	858	1683	2385	3066	3708	4057	4279	4486	4644	3241
T5	858	1641	2311	2998	3676	4015	4200	4386	4479	3174
T6	858	1467	2068	2737	3395	3622	4037	4180	4295	2962
KattiKhel Soil										
T7	858	1716	2547	3283	3924	4259	4494	4652	4742	3386
T8	858	1716	2547	3299	3969	4140	4331	4479	4581	3324
T9	858	1716	2547	3317	3940	4371	4612	4756	4860	3442
T10	858	1716	2547	3317	4015	4342	4598	4727	4824	3438
T11	858	1716	2547	3932	3970	4357	4595	4791	4899	3518
T12 (Control)	858	1716	2547	3303	3932	4347	4487	4681	4256	3347

*For details of treatment, see footnote of Table I.

production ranged from 47 to 158 mg CO_2 kg^{-1} soil hr^{-1} in the pesticide amended soil, while CO_2 production was 54 mg CO_2 kg^{-1} soil hr^{-1} in control soil. The rate of CO_2 evolution in the KattiKhel series ranged from 97 to 108 mg CO_2 kg^{-1} soil hr^{-1} in the pesticides amended soil, which is slightly lower than that of control soil (194 mg CO_2 kg^{-1} soil hr^{-1}).

The CO_2 production decreased in all treatments at the end of incubation compared to that of day 1. Based on these findings, no appreciable effect was observed on the rate of CO_2 production after application of selected pesticides.

Cumulative CO_2 production

The effect of pesticides on cumulative CO_2 production was measured in two series of the soil samples during different incubation periods. Amount of CO_2 produced during 15 days of incubation varied greatly with the pesticides treatments. The amount of cumulative CO_2 in the MCPA (Chwastox) was 3241 mg $kg^{-1}$of soil which was the highest amount of CO_2 produced in the Pirsabak series (Table II). The lowest CO_2 (2962 mg kg^{-1}) was produced after the Glyphosate treatment, followed by 3174 mg kg^{-1} after 2, 4-D treatment, 3167 mg kg^{-1} in Trifluralin treatment, 3118 mg in the Pendimethaluin treatment. Similarly, the cumulative CO_2 production was 3442 mg kg^{-1} in the MCPA (Chwastox) treated soil of the KattiKhel series, while the the lowest amount (3324 mg kg^{-1}) of CO_2 was produced in the Pendimethalin treatment, followed by Trifluralin (3386 mg kg^{-1}), 2,4-D (3438 mg kg^{-1}) and Glyphosate (3438 mg kg^{-1}) treatments. These results indicated that all the pesticides (except MCPA (Chwastox)) could be toxic to soil microbes in both soils (Pirsabak and KattiKhel). It is evident from literature that some pesticides were highly toxic to soil microorganisms and inhibit their biochemical activities, while others might be less toxic and could be easily degraded by microorganisms (Gao*et al.*, 2013; Muñoz-Leoz *et al.*, 2013).

CONCLUSIONS

In this study we have evaluated five commonly used pesticides (Pendimethalin, Trifluralin, MCPA (Chwastox), Glyphosate, and 2, 4-D) for their effects on microbial activities in agriculture soils from District Charsadda. It has been concluded that in both series of soils (Pirsabak and KattiKhel), all the pesticides used were highly toxic to soil microbes, as evidenced by the suppression of CO_2 produced.

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