Effect of pesticides on soil microorganisms

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Abstract. In light of the rapidly growing human population, extensive pesticides have been utilized to maximize crop production. This has become a major environmental concern. To assess the influence of commonly used pesticides on soil microorganisms counts and microbial activities in the form of CO₂ production, a factorial experiment was conducted. Herbicide (Glyset I.P.A, Glyphosate 48%) and insecticides Miraj (Alphacypermethrin 10%) and Malathion (50% WP) were separately added to the soil at 0, 50, 100 and 200 ppm and incubated in the laboratory at 30 °C. The counts of bacteria, fungi, actinomycetes and CO₂ production were examined weekly for 7 consecutive weeks. The results demonstrated that the addition of the three mentioned pesticides significantly decreased the microbial activities and counts of soil bacteria, fungi and actinomycetes. The observed effect was dependent upon the type and amount of pesticide as well as the length of incubation period. The microbial activities and the number of bacteria, fungi, and actinomycetes were inversely proportional to the concentration of pesticides added to the soil. In most treatments, soil samples treated with 200 ppm of Malathion demonstrated the lowest microbial activities and counts of bacteria, fungi and actinomycetes. This study suggests that the investigated pesticides negatively affect microbial counts and activity in soil, which confirms and reinforces previously reported environmental concerns.

Key words: soil, pesticides, herbicide, insecticide, bacteria, fungi, actinomycetes

1. Introduction

Due to rapidly growing human population, extensive pesticides have been utilized to maximize crop production. The extensive consumption of pesticides in cultivated soils leads to the pollution of the soil with harmful materials (Muñoz-Leoz et al., 2013). About 3 million tons of pesticides that costs about US$ 40 billion is utilized in world agriculture annually (Pan UK, 2003). About 99.9% from the applied pesticide not reached to target organisms and become as pesticide residues accumulation which pollute the soil environment and just 0.1% reached to target organisms (Carriger et al., 2006; Pimental, 1995). Both pesticides residues accumulation and microorganisms activity usually present in the same reign, soil top layer (Harris and Sans, 1969; Alexander, 1961). The impact of different pesticides on the growth of soil microorganisms and its activity are difficult to expect. Even if the pesticides used in low concentration they effect chemical and biological properties, biochemical activity and soil microorganisms (Cycon et al., 2006; Singh et al., 2008; Cycon et al., 2010).
Pesticides in the soil impact the non target and useful microorganisms (Singh and Prasad, 1991; Bhuyan et al., 1992) and their activities (Schuster and Schroder, 1990). Beneficial soil microorganisms play essential role in soil fertility and productivity such as organic matter biodegradation, nutrients recycling, humus formation, Soil structural stability, nitrogen fixation, plant growth promotion, disease biocontrol, and other biochemical transformation such as ammonification, nitrification phosphorus solubilizing (Prasad Reddy et al., 1984; Husain et al., 2003). The effect of pesticides on soil microorganisms and their activity depend upon the type of pesticides used, quantities and soil conditions (Subhani et al., 2000). The objective of this study is to assess the influence of three usually used pesticides on soil microorganisms counts and microbial activities in the form of CO2 production.

2. Material and Methods

2.1. Soil sampling and analysis

The soil samples were taken from Alrashedia area 5 km north of Mosul city. The soil was taken from surface area (0-20) cm. To remove debris, the soil was sieved with 2 mm sieve. The physical and chemical characteristics of the soil were determined as following. Soil texture by hydrometer method, Soil Reaction (pH) by glass electrode method (1:2.5 soil water suspension), soluble salts by Electrical Conductivity, Organic material by rapid titration method (Black et al., 1965), available phosphorus by Olsen’s method (Olsen et al., 1954) Ca and Mg by Graham method (Graham et al., 1962), Potassium and sodium by flame photometer, (Jackson, 1973). Some of soil physical and chemical properties were recorded in Table 1.

| Characteristics | pH (µS/cm.) | Organic matter (%) | Available Phosphorus (mg/L) | Ca (meq/L) | Mg (meq/L) |
|-----------------|-------------|-------------------|-----------------------------|-----------|------------|
| Results         | 7.8         | 99                | 1.08                        | 26        | 98         |
| K Characteristics (meq/L) | SO₄ | Clay (%) | Silt (%) | Sand (%) | Soil Texture |
| Results         | 122         | 25.8              | 14.77                       | 53.08     | 32.58      |

2.2. Soil sample preparation and Experimental design

200 gm of sieved soil was placed in 250 ml flask. The treatments involved of three pesticides. Herbicide (Glyset I.P.A, Glyphosate 48%) and two insecticides Miraj (Alphacypermethrin 10%) and Malathion (50% WP). pesticides were applied at 0, 50, 100, 200 ppm levels. The pesticides pollution include twelve treatments in a completely randomized design replicated three times. All components were gently mixed with the soil. The moisture content of soil was got to 60% water holding capacity. Distilled water was added to maintain them 60% of WHC. For measuring CO₂ production, 10 ml of 2M sodium hydroxide solutions was placed in a glass tube and put the tube gently on soil surface in each flask. The flasks were closed well with rubber stoppers to avoid any gaseous exchange between the flasks and outside atmosphere. A blank, in three replicate, was also done to account for the quantity of CO₂ already present in the flask’s atmosphere. The flasks were incubated at a temperature of 30C. Soil sampling was conducted weekly intervals for 7 weeks.
2.3. Measurement of microbial activity

activities of microorganisms were determined in the form of carbon dioxide production according to Anderson et al., 1982. The glass tube was gently got out of flask weekly and the sodium hydroxide solution was transmitted to clean flask. For following incubation fresh sodium hydroxide solution was put in clean glass tube and placed in the same flask and it is gave back to the incubator. The process was reiterate at the finish of each former incubation period. After addition 10ml of 1M barium chloride solution and drops of phenolphthalein, to the recovered sodium hydroxide solution and titrated against 1 M hydrochloric acid solution till the pink color is gone. During the reaction one mole of carbon dioxide equalize two moles of sodium hydroxide. The quantity of released CO$_2$ was adjusted as mg CO$_2$/100g soil

\[2\text{NaOH} + \text{CO}_2 \rightarrow \text{Na}_2\text{CO}_3\]

\[\text{Na}_2\text{CO}_3 + \text{BaCl}_2 \rightarrow 2\text{NaCl} + \text{BaCO}_3\]

2.4. Microbial analysis

In assessing microbial population, standard plate count methods were used to prepare nutrient agar (NA) for assessment of the bacteria population, potato dextrose agar (PDA) for assessment of fungi, Starch casein nitrate (SCN) agar - for assessment of actinomycetes. One gram each of the soil samples were measured into the test tube containing 9 ml sterile distilled water and serially diluted to dilution factor $10^5$ and 1 ml of the proper dilutions was pipette into sterile plate with appropriate medium which were incubated at 30°C. All plated were incubated inverted wise. Microbial counted were done at 48 hours for NA and 72 hours for PDA and 6 days for(SCN) (Stanley,2015; Adesina and Adelasoye, 2014)

2.5. Statistical analysis

ANOVA was carried out. The means were compared using Least Significant Difference LSD test at p <0.05 after, ANOVA.

3. Results and Discussion

3.1. Effect of pesticides on bacteria count

Table 2 shows the addition of pesticides decreased the count of bacteria in all pesticides types and at all incubation periods. In the first week of incubation period, the addition of Glyset (Glyphosate 48%) 50ppm, 100ppm and 200ppm decreased the count of bacteria by 4%, 11% and 13% respectively. While at 7th week of incubation, the reduction in bacteria count was 6%, 9% and 9% respectively. This depression was significant just at 100 ppm and 200 ppm. This results are consistent with (Newman et al., 2016; Aralujo et al.,2003) who concluded that the presence of glyphosate decreased the number of bacteria, microbial biomass and acidobacteria population. They believed the reduction in the bacteria population for a long time could weaken some biogeochemical reactions accomplished by these microorganisms. (Grossbard and Atkinson, 1985) reported that the toxic effects of pesticides as result of inhibition of amino acid synthesis via the shikimic acid pathway. Conversely, other studies have demonstrated significant increase in bacteria count after glyphosate treatments (Partoazar et al., 2011; Wardle and Parkinson,1990).

Our results in table 2 also show that the addition of Miraj ((Alphacypermethrin 10%) insecticide decreased the population of bacteria. In the first week of incubation period, the addition of Miraj
50ppm, 100ppm and 200ppm decreased the count of bacteria by 18%, 24% and 32% respectively. While at 7th week of incubation, the reduction in bacteria count was 9%, 17% and 45% respectively. Similar results were observed by (Goswami et al., 2013; Wesley et al., 2017) who reported that the decrease in the soil microbial count and biomass can be associated with the toxic effect of Cypermethrin on soil microorganisms. The presence of Cypermethrin and thiamethoxam inhibited the metabolic process and significantly decreased ammonifying, nitrifying and denitrifying bacteria compared to the untreated sample (Nicoleta et al., 2015).

The results in table 2 show that the presence of malathion insecticide decreased bacteria number. In the first week of incubation period, the addition of malathion 50ppm, 100ppm, and 200ppm decreased the number of bacteria 40%, 42% and 59% respectively. While at 7th week of incubation period, the reduction was 32%, 38% and 41% respectively. These results agree with (Haleem et al., 2013; Gonzalez Lopez et al., 1993) who concluded the presence of malathion significantly decreased the population of bacteria.

**Table 2. Effect of pesticides on Bacteria count x 10^5**

| Treatments           | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|----------------------|-------|-------|-------|-------|-------|-------|-------|
| (Unamended Control)  | 73.22 | 70.2  | 66.11 | 65.22 | 62.44 | 59.65 | 57.44 |
| Glyset 50 ppm        | 70.12 | 66.11 | 63.48 | 61.22 | 59.11 | 58.05 | 54.10 |
| Glyset 100 ppm       | 65.18 | 58.45 | 59.58 | 55.14 | 55.33 | 54.25 | 52.11 |
| Glyset 200 ppm       | 59.62 | 55.20 | 54.10 | 56.67 | 56.55 | 55.59 | 52.33 |
| Miraj 50 ppm         | 60.11 | 55.12 | 50.22 | 55.34 | 53.55 | 55.11 | 52.22 |
| Miraj 100 ppm        | 55.39 | 52.00 | 50.22 | 51.32 | 44.59 | 46.91 | 47.39 |
| Miraj 200 ppm        | 50.11 | 48.29 | 46.11 | 47.15 | 40.48 | 45.49 | 31.40 |
| Malathion 50 ppm     | 44.22 | 40.59 | 38.66 | 36.88 | 36.26 | 40.16 | 38.84 |
| Malathion 100 ppm    | 42.44 | 40.38 | 39.36 | 35.65 | 34.22 | 36.40 | 35.64 |
| Malathion 200 ppm    | 30.11 | 38.22 | 32.48 | 31.11 | 30.32 | 32.87 | 33.84 |
| LSD                  | 6.48  | 7.22  | 6.51  | 5.57  | 4.80  | 4.40  | 4.51  |

Table 3 shows that the presence of glyset herbicide decreased fungi count in all glyset concentration. However, the reduction in fungi count was just significant at 200ppm. The addition of glyset 200ppm, decreased the fungi count by 20% and 13% at first week of incubation period and 7th week respectively. Tanney and Hutchison, 2010 reported that the addition of glyphosate depressed the growth of 21 from 22 fungal species.

Out results in table 3 show that the presence of Miraj insecticide reduced the fungi count at all concentrations and period of incubations. The addition of Miraj at 50ppm, 100ppm and 200ppm, decreased the fungi count in the first week of incubation 60%, 61% and 63% respectively. While the reduction at 7th week was 48%, 50% and 62% respectively. Goswami et al., 2013 concluded that cypermethrin application had toxic effects on soil microorganisms.

Table 3 shows the addition of malathion decreased the fungi counts at all concentrations and periods. During the first week of incubation, the addition of malathion at 50ppm, 100ppm and 200ppm decreased fungal population 56 %, 62% and 66% respectively. While during the 7th week, the reduction was 58%, 64% and 65% respectively. Similarly, studies have shown that the presence of malathion insecticide decreased fungal population (Walia et al., 2018; Smith, et al., 2000). Our results show that the most adverse effect was seen in soil treated with malathion specially at 200ppm.
The effect of pesticides on actinomycetes population was shown in Table 4. The presence of glyset herbicide inhibited actinomyces population. During the first week of incubation, the addition of glyset 50ppm, 100ppm and 200ppm declined actinomycetes population by 5%, 7% and 22% respectively. However, during the 7th week, the reduction was 7%, 9% and 10% respectively. Our results show that the reduction of actinomycetes population as a result of glyset addition was significant just at 200ppm.

Table 4 shows actinomycetes population was decreased as a result of Miraj insecticide addition. During the first week of incubation, the depression in actinomycetes population was 54%, 56% and 60% as a result of addition of miraj insecticide 50ppm, 100ppm and 200ppm respectively. During the 7th week, the depression was 63%, 64% and 69% respectively.

Actinomycetes population was depressed in the soil treated with malathion (Table 4). During the first week of incubation period, the addition of malathion 50ppm, 100ppm and 200ppm decreased actinomycetes population by 34%, 36% and 40% respectively. While during the 7th week, the reduction in actinomycetes population was 37%, 42% and 50% respectively. Our results show that the most adverse effect was seen in soil treated with malathion specially at 200ppm. These results are coordinated with several studies (Walia et al., 2018; Haleem, et al., 2013) who reported that the population of actinomycetes decreased by malathion treatment.

### Table 3. Effect of pesticides on fungi count x 10^3

| Treatments                  | Incubation periods/ weeks |
|-----------------------------|---------------------------|
| (Unamended Control)         | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| Glyset 50 ppm               | 23.11 | 22.32 | 23.68 | 20.11 | 18.19 | 19.68 | 18.88 |
| Glyset 100 ppm              | 22.19 | 20.68 | 22.67 | 18.62 | 17.28 | 18.89 | 17.74 |
| Glyset 200 ppm              | 21.11 | 19.66 | 22.22 | 17.89 | 16.00 | 18.49 | 17.37 |
| Glyset 50 ppm               | 18.51 | 17.76 | 19.88 | 16.22 | 15.45 | 17.30 | 16.38 |
| Miraj 50 ppm                | 9.20 | 10.33 | 9.42 | 9.38 | 11.12 | 11.34 | 9.88 |
| Miraj 100 ppm               | 9.06 | 10.20 | 9.11 | 8.78 | 10.11 | 9.33 | 9.48 |
| Miraj 200 ppm               | 8.66 | 7.22 | 6.88 | 7.11 | 7.38 | 7.22 | 7.11 |
| Malathion 50 ppm            | 10.11 | 11.12 | 10.88 | 9.39 | 10.11 | 8.88 | 7.98 |
| Malathion 100 ppm           | 8.88 | 7.48 | 7.66 | 8.11 | 7.22 | 7.48 | 6.88 |
| Malathion 200 ppm           | 7.88 | 5.98 | 5.56 | 6.11 | 6.38 | 6.12 | 6.55 |
| LSD                         | 3.48 | 2.81 | 3.21 | 2.88 | 2.52 | 2.22 | 1.86 |

### Table 4. Effect of pesticides on Actinomycetes count x 10^3

| Treatments                  | Incubation periods/ weeks |
|-----------------------------|---------------------------|
| (Unamended Control)         | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| Glyset 50 ppm               | 140.21 | 140.14 | 137.12 | 121.44 | 124.22 | 120.44 | 121.66 |
| Glyset 100 ppm              | 133.42 | 133.00 | 130.15 | 112.53 | 117.8 | 114.32 | 113.55 |
| Glyset 200 ppm              | 130.88 | 131.22 | 124.67 | 115.64 | 116.6 | 112.12 | 111.22 |
| Glyset 300 ppm              | 109.22 | 107.22 | 123.30 | 100.43 | 105.55 | 109.2 | 109.52 |
| Miraj 50 ppm                | 65.12 | 61.42 | 59.44 | 60.22 | 46.22 | 45.44 | 45.22 |
| Miraj 100 ppm               | 61.46 | 58.22 | 57.22 | 55.11 | 45.44 | 43.22 | 44.12 |
| Miraj 200 ppm               | 55.42 | 52.42 | 52.42 | 50.66 | 40.32 | 40.66 | 38.18 |
| Malathion 50 ppm            | 92.22 | 88.68 | 93.44 | 85.56 | 80.22 | 78.22 | 77.22 |
| Malathion 100 ppm           | 90.42 | 85.22 | 88.22 | 80.34 | 76.11 | 72.44 | 70.42 |
| Malathion 200 ppm           | 83.44 | 75.11 | 79.14 | 72.44 | 69.12 | 66.48 | 60.44 |
| LSD                         | 12.22 | 15.22 | 17.12 | 14.22 | 11.22 | 11.52 | 10.82 |
3.2. Microbial activity

The pesticides treatment utilized in the current study caused adverse impact on the microbial activity in the form of CO$_2$ production (table 5). There were significant decreases in CO$_2$ production and these decreases significant in all pesticides types and concentrations except Glyset 50 ppm, 100 ppm and miraj 50 ppm. The addition of 200ppm glyset decreased the CO$_2$ production during first and 7th week of incubation by 18% and 26% respectively. During the first week, the addition of Miraj insecticide 100ppm and 200ppm, decreased CO$_2$ production by 25%, 29% respectively. While the reduction during the 7th week was 32% and 36% respectively. The most adverse effect was seen with soil treated with malathion insecticide. During the first week of incubation, the addition of malathion 50ppm, 100ppm and 200ppm depressed CO$_2$ production by 31%, 37% and 43% respectively. While the depression in CO$_2$ production during 7th week was 42%, 45% and 52% respectively. The same results were shown by Goswami et al., 2013 who reported that the application of cypermethrin insecticide on soil at high concentration leads to poisonous impact on soil biomass, respiration and FDHA activity. Yousaf et al., 2013 concluded that the pesticides were very poisonous to soil microbes, as showed by the decrease of CO2 produced.

Table 5. Effect of pesticides on CO2 evolution (mg CO2 100g soil) during different incubation periods

| Treatments                  | Incubation periods/ weeks |
|-----------------------------|---------------------------|
| (Unamended Control)         | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Glyset 50 ppm               | 140 | 122 | 111 | 118 | 116 | 120 | 117 |
| Glyset 100 ppm              | 131 | 115 | 106 | 119 | 110 | 114 | 109 |
| Glyset 200 ppm              | 127 | 110 | 119 | 115 | 107 | 112 | 110 |
| Miraj 50 ppm                | 115 | 88 | 85 | 89 | 91 | 92 | 87 |
| Miraj 100 ppm               | 126 | 120 | 122 | 106 | 110 | 111 | 109 |
| Miraj 200 ppm               | 105 | 102 | 96 | 98 | 88 | 84 | 80 |
| Malathion 50 ppm            | 100 | 100 | 90 | 88 | 80 | 76 | 75 |
| Malathion 100 ppm           | 96 | 88 | 80 | 76 | 72 | 70 | 68 |
| Malathion 200 ppm           | 88 | 84 | 78 | 74 | 70 | 64 | 64 |
| LSD                         | 80 | 66 | 60 | 64 | 62 | 60 | 56 |

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