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Effect of Malathion and Carbaryl Insecticides on Agriculture Soil Quality

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ABSTRACT

Human activities as agriculture and industry are among soil pollution sources by pesticides and fertilizers. Repeated application of pesticides may cause many serious effects on human health and environmental compartments, as a part soils. This study was carried out to determine the effects of malathion and carbaryl insecticides at doses: recommended (R), double-fold (2R), five-fold (5R) and ten-fold (10R) on soil bacterial population, dehydrogenase enzyme activity and soil basal respiration rate which may ultimately contribute threats to soil ecosystems if they do not degrade. The observed data suggested that 10-fold dose of carbaryl cause proliferation of bacterial population in soil to $36.97 \text{ CFU} \times 10^5 \cdot \text{g}^{-1}$ dry soil in comparing with control. Highest dehydrogenase and basal respiration rate were $62.34 \text{ TPF } \mu\text{g} \cdot \text{ml}^{-1} \cdot 24 \text{ h}^{-1}$ and $0.04 \text{ BAS } \mu\text{gCO}_2\text{-C} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ detected after malathion application at its 10-fold dose. Positive correlations among bacterial population, dehydrogenase activity and basal respiration rate were observed after malathion application, whereas negative correlations among these parameters were detected after carbaryl application.

Keywords: Soil Pollution; Malathion; Carbaryl; Dehydrogenase; Bacterial Population; Respiration Rate.

1. INTRODUCTION

Soil is an important natural resources, and the entrance of materials will cause changes in soil quality. This problem causes soil to remove from its natural state (Khakbaz *et al.*, 2012). Thus the undesirable changes in soil's physical, chemical or biological characteristics known as pollution that harmfully affect the organism's life (Khudhur and Sarmamy, 2019). The term pesticide covers a wide range of compounds that used to kill, extinct or control of pests in the line of agricultural and environmental field such as human house and garden (Usman, 2018). The extensive use of the insecticides cause pollution of soil and water systems and constitute potential environmental and human health hazards (Afify *et al.*, 2010).

Human exposure to carbamate insecticides occurs via contaminated food or other routes and because of their potential human risk, many studies have analyzed the presence of carbaryl in fruits and food products (Glatfelter *et al.*, 2021). Carbaryl is one of the most widely used broad spectrum insecticides in agriculture, professional turf management, professional ornamental production, and in the residential lawn and garden markets. Carbaryl (1-naphthyl N-methyl carbamate), broad-spectrum insecticide, both contact and systemic, against over 150 major pests is also extensively used in the tropics and sub-tropics at rates ranging from 0.57 to $4.5 \text{ kg a.i. ha}^{-1}$ for controlling rice brown plant hopper (Megharaj *et al.*, 1989). The most of the toxicological studies

were conducted in laboratory animals; however, studies are available that involved direct dosing of humans with carbaryl. Carbaryl is highly toxic and the United States Environmental Protection Agency (EPA) has classified carbaryl insecticide as probable human carcinogens (Gunasekara *et al.*, 2008). Malathion [(dimethoxyphosphorothioyl)sulfanyl]butanedioate, Diethyl) is an insecticide in the chemical family known as organophosphates. Products containing malathion are used outdoors to control a wide variety of insects in agricultural settings and around people's homes. Malathion has also been used in public health mosquito control and fruit fly eradication programs. Malathion may also be found in some special shampoos for treating lice. Malathion was first registered for use in the United States in 1956. Products containing malathion may be liquids, dusts, wettable powders, or emulsions (Gervais *et al.*, 2009).

Soil enzymes are the key players in biochemical process of organic matter recycling in the soil system and can be either extracellular or intracellular. Intracellular enzymes are found in cell's cytoplasm or bound to the cell walls of living cells, whereas, extracellular enzymes released into the soil (Jat *et al.*, 2021). Soil dehydrogenases consists in the biological oxidation of organic matter in the soil by hydrogen transfer from the organic substrate to inorganic acceptors (Zhang *et al.* 2010).

Bacteria are the major class of microorganisms that keep soils healthy and productive. They are among the non-target organisms that expose to the undesirable effect of residual pesticides which cause a variety of acute and chronic toxicity such as reducing their numbers, biochemical activity, diversity and changing their community structures. Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms (Mawlood and Khudhur, 2020).

Soil respiration refers to the production of carbon dioxide when soil organisms respire. This includes respiration of plant roots, the rhizosphere, microbes and fauna. Soil respiration is a key ecosystem process that releases carbon from the soil in the form of CO₂. CO₂ is acquired by plants from the atmosphere and converted into organic compounds in the process of photosynthesis. Plants use these organic compounds to build structural components or respire them to release energy. When plant respiration occurs below-ground in the roots, it adds to soil respiration. Over time, plant structural components are consumed by heterotrophs. This heterotrophic consumption releases CO₂ and when this CO₂ is released by below-ground organisms, it is considered soil respiration (Jukka *et al.*, 2010). Due to over and repeated applications of pesticides in the environment, many undesirable effects may occur to the environment and human health. Thus the

main aim of this study is to determine the effects of malathion and carbaryl insecticides on soil bacteria, dehydrogenase activity and basal respiration rate.

2. MATEREAL AND METHODS

2.1. Experimental layout

By this study, a sandy-loam soil was used, screened from gravel and stones, air-dried and 2-mm sieved. A laboratory experiment was designed including the study of the effects of two insecticides including Carbaryl and Malathion each with four treatments. For comparison a control soil (without insecticide treatment) was used. The experimental containers were packed with 100 g of soil.

2.2. Pesticide preparation and soil treatment

Four different doses of both Carbaryl and Malathion (R, 2R, 5R and 10R) were prepared separately according to their commercial ingredients on the insecticide sheet. Then the soil samples were mixed with each insecticide doses separately inside sealed glass containers to obtain homogenous distributions. Then the pesticide-treated soils were sealed and left for an overnight at room temperature.

2.3. Counting of bacterial population

Total bacterial population count was performed by standard plate technique. Soil samples were serially diluted from 10^{-1} until 10^{-6} in aseptic condition and one ml of each dilution was poured into plates containing nutrient agar and pre-labelled for each insecticide dose, then all the plates were incubated at $35\text{ }^{\circ}\text{C}$ for 24-48 hrs and taking 30-300 colonies per plate into account (Harley and Prescott, 2002). Colony Forming Units (CFU) were estimated by using the below formula as given by (Aneja, 2003).

$$\text{Number of CFU/ml} = \frac{\text{Number of colonies}}{\text{sample size} \times \text{dilution factor}}$$

2.4. Estimation of soil dehydrogenase activity

Dehydrogenase activity was estimated according to the modified method of Casida 1977 described by (Mawlood and Khudhur 2020). Soil samples were mixed with CaCO_3 and 3% aqueous solution of triphenyl tetrazolium chloride (TTC). The produced triphenyl tetrazolium formazone product was measured spectrophotometrically at 485 nm. The activities were expressed as $\mu\text{g TPF}\cdot\text{g}^{-1}\text{ dry soil}\cdot 24\text{h}^{-1}$.

2.5. Determination of basal soil respiration

Soil respiration was detected according to (Khopkar, 1998). CO_2 was released and absorbed into NaOH. One hundred grams of soil was placed into sealed glass containers and NaOH (0.05M) inside small flasks was placed inside the soil. After 3 days, titration was done by adding 2ml of BaCl_2 and 1-2 drops of phenolphthalein indicator to the flasks containing NaOH, then titrated against 0.1M HCL until the color changed from pink to colorless. Soil respiration was calculated according to the below equation:

$$R_{CO_2} = \frac{2,2 (V_b - V_p)}{m_s w_{sd}}$$

Where:

R_{CO_2} is the rate of CO_2 evolution on a dry soil ($mgCO_2 g^{-1}$).

V_b is the volume of HCl consumed in the control (ml).

V_p is the volume of HCl consumed in the test sample (ml).

m_s is the mass of the soil sample (g).

2,2 is a factor (1 ml of 0,1 molar HCl corresponds to 2,2 mg of CO_2) ($mg ml^{-1}$). In appendix 1 this factor is explained.

w_{sd} is the dry mass fraction of the soil.

2.6. Data display

The obtained data during the present study was graphically analysed using Microsoft Excel 2019. All data expressed as mean values. Pearson correlation among bacterial population, dehydrogenase activity and basal respiration rate was performed by using SPSS version 26.

3. RESULT AND DISCUSSION

3.1 Total bacteria

According to (Figure 1), the highest bacterial count ($36.97 CFU \times 10^5.g^{-1}$ dry soil) was observed in Carbaryl 10R treatment followed by Carbaryl 5R treatment in which bacterial count was ($12.97 CFU \times 10^5.g^{-1}$ dry soil) and the lowest bacterial count ($6.28 CFU \times 10^5.g^{-1}$ dry soil) was observed in control, this indicate that Carbaryl insecticide application to soil cause increasing in bacterial count after two weeks of its degradation and this may refer to the ability of bacteria to use a fraction of the used pesticide as growth C source, energy and nutrients (Johnsen *et al.*, 2001 and Kalia and Gosal, 2011). So, it has been found that the half-life of carbaryl in sandy loam soil was 7-14 days, whereas, malathion half-life at its technical grade added to a sandy loam soil degraded with a half-life of approximately 2.5 days (USEPA, 2006). Moreover, it is well documented that with certain pesticides, repeated applications can promote microbial populations capable of selectively degrading that pesticide and various pesticides may degrade by bacterial isolates (Kanekar *et al.*, 2004).

Moreover, pearson correlation showed positive correlations among bacterial population, dehydrogenase activity and basal respiration rate regarding to malation effects, whereas effects of carbaryl was showed negative correlations (Table 1).

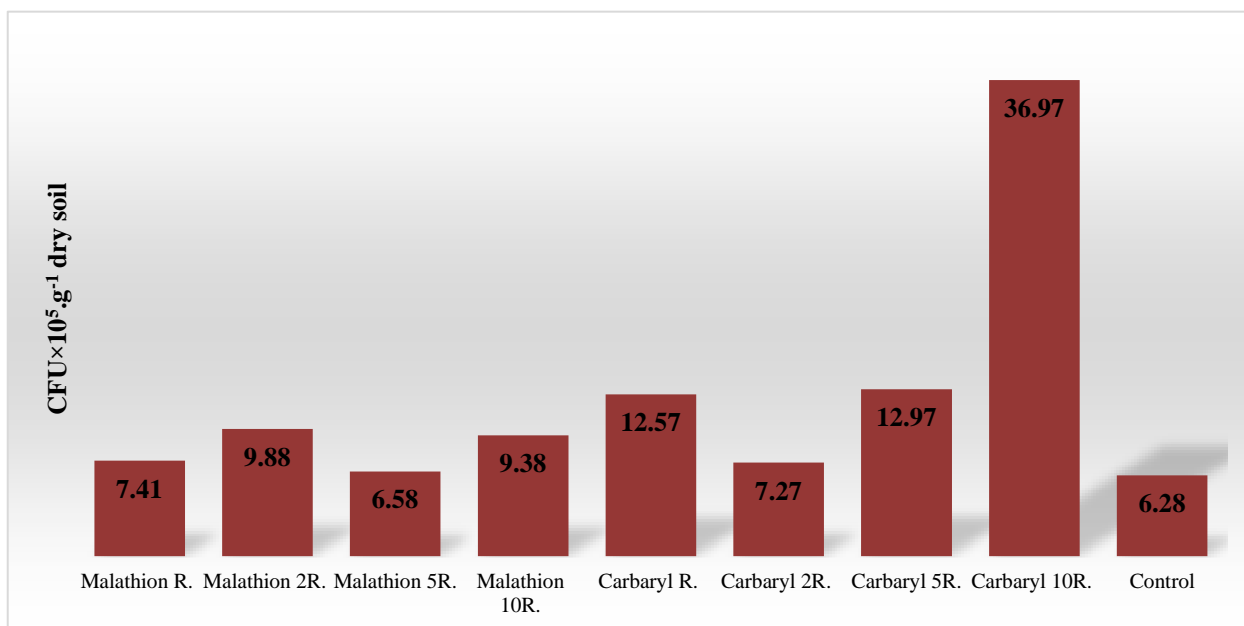


Figure 1: Total bacterial population in soil samples two weeks after insecticide application.

Table 1: Pearson correlation among bacterial population, dehydrogenase activity and basal respiration rate followed insecticide application.

Parameters	Malathion			Carbaryl		
	Bacterial population	Dehydrogenase activity	Basal respiration rate	Bacterial population	Dehydrogenase activity	Basal respiration rate
Bacterial population	1	0.553	0.285	1	-0.335	-0.429
Dehydrogenase activity		1	.917*		1	0.519
Basal respiration rate			1			1

* Correlation is significant at the 0.05 level (2-tailed).

3.2 Dehydrogenase enzyme

Soil enzymes respond quickly to changes in natural and anthropogenic factors that affect soil and many researchers investigated their response to environmental pollutants like pesticides (Sardar and Kole, 2005). Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Liu *et al.*, 2008). Data of (Figure 2) shows dehydrogenase activity in soil samples two weeks after insecticide application. According to (Figure 3), as malathion dose increased, dehydrogenase activity also increased and the highest dehydrogenase was detected in malathion 10R which was 62.34 TPF $\mu\text{g}\cdot\text{ml}^{-1}\cdot 24\text{ h}^{-1}$. Whereas, dehydrogenase activity was decreased as carbaryl doses were increased (Figure 4), so ten-fold dose of carbaryl produced the lowest dehydrogenase activity 5.74 TPF $\mu\text{g}\cdot\text{ml}^{-1}\cdot 24\text{ h}^{-1}$. The reported sensitivity of dehydrogenase to the adverse effects of pesticides is consistent with the study of Cycon *et al.* (2005) and Menon *et al.* (2005).

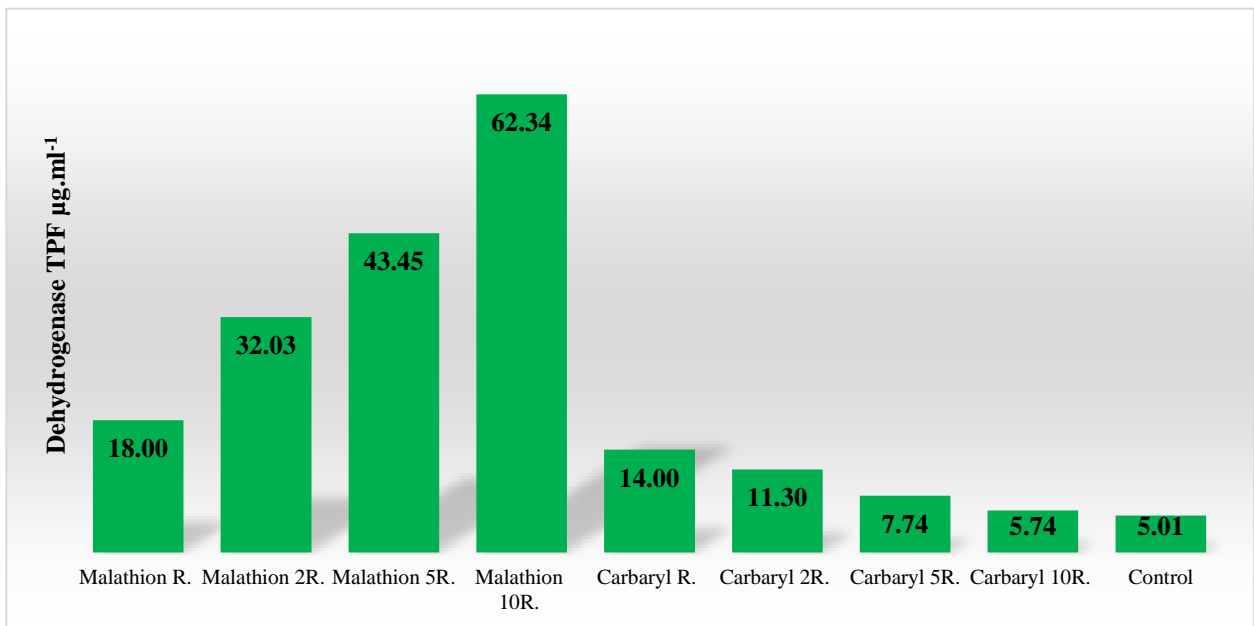


Figure 2: Dehydrogenase activity in soil samples two weeks after insectide application.

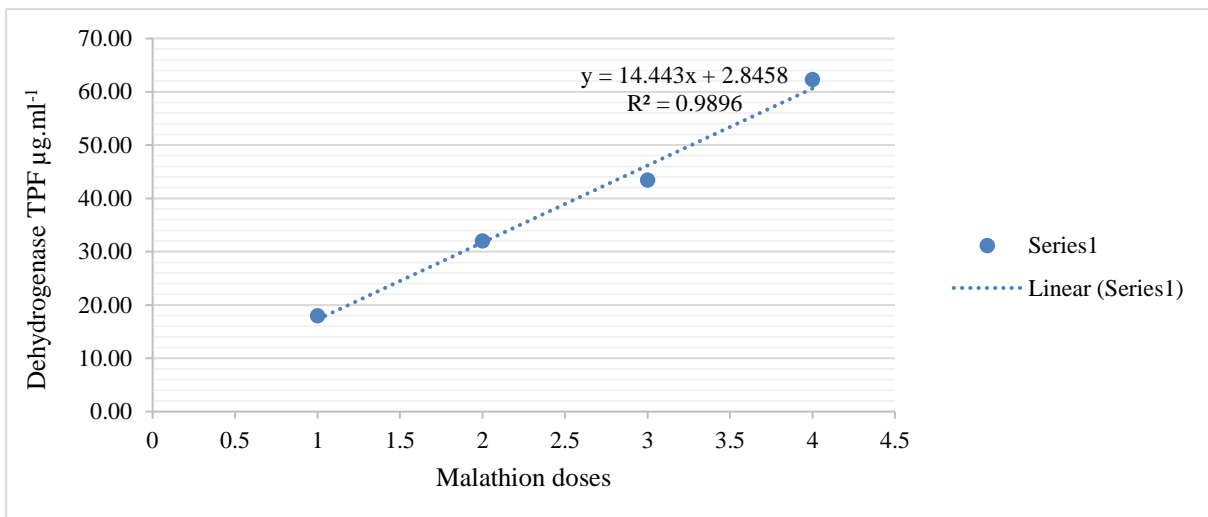


Figure 3: Correlation between malathion doses and dehydrogenase activity.

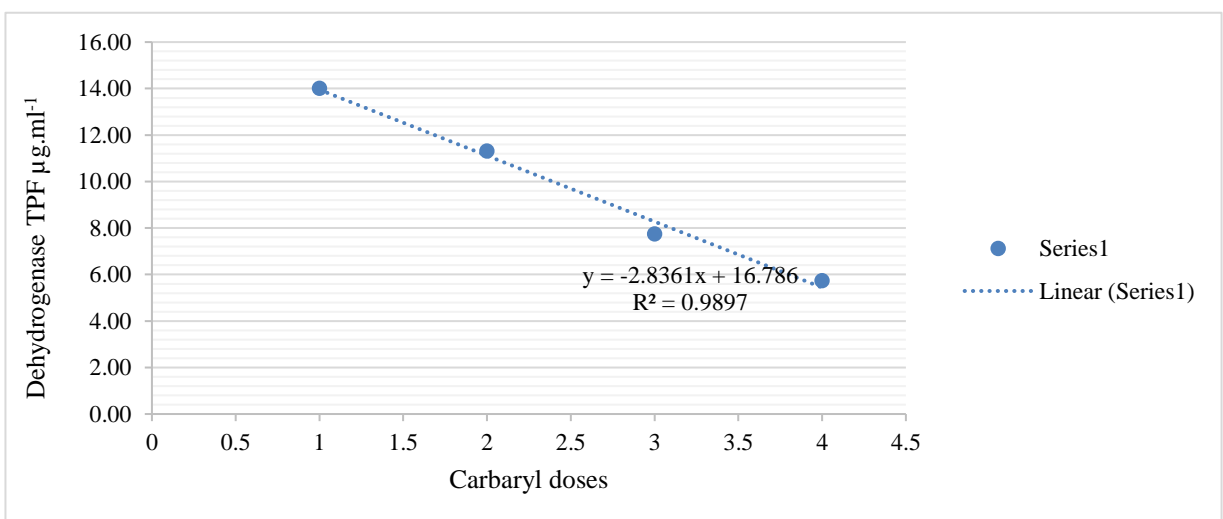


Figure 4: Correlation between carbaryl doses and dehydrogenase activity.

According to (Table 1), a significant positive correlation was observed between dehydrogenase and soil respiration rate by r value of 0.917* after malathion treatment. As well as the correlation between dehydrogenase and soil respiration rate was also positive following carbaryl treatment.

3.3 Basal soil respiration

Based on (Figure 5) data, soil respiration rate increased with increasing insecticide doses except for carbaryl 10-fold dose which caused a shift in soil respiration. The highest soil respiration rate was observed in both malathion 10R and carbaryl 5R which was 0.04 BAS $\mu\text{gCO}_2\text{-C.g}^{-1}\text{.h}^{-1}$. In comparing with control, soil respiration rate was increased with response to malathion and carbaryl application regardless to their doses and this an indication of the conversion of nutrients in organic matter to forms available for plant use e.g., phosphate as PO_4 , nitrate nitrogen as NO_3 , and sulfate as SO_4 (Mawlood and Khudhur, 2020).

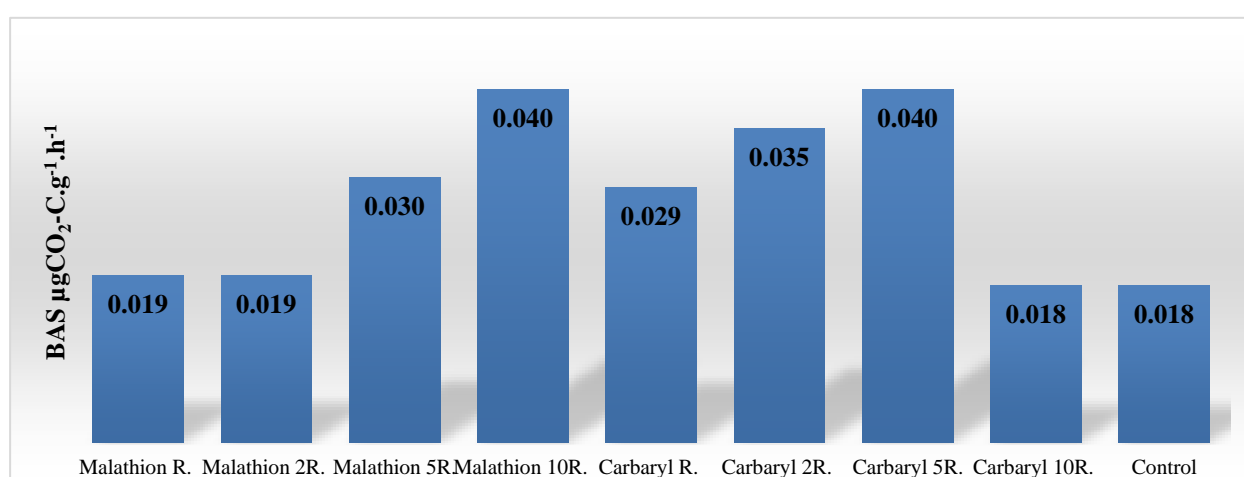


Figure 5: Basal respiration in soil samples two weeks after insecticide application.

A significant positive correlation was observed between soil respiration rate and dehydrogenase activity by r value of 0.917* after malathion treatment (Table 1). As well as the correlation between soil respiration rate and dehydrogenase activity was also positive after carbaryl treatment. Furthermore, increasing malathion doses caused increasing in soil respiration rate (Figure 6).

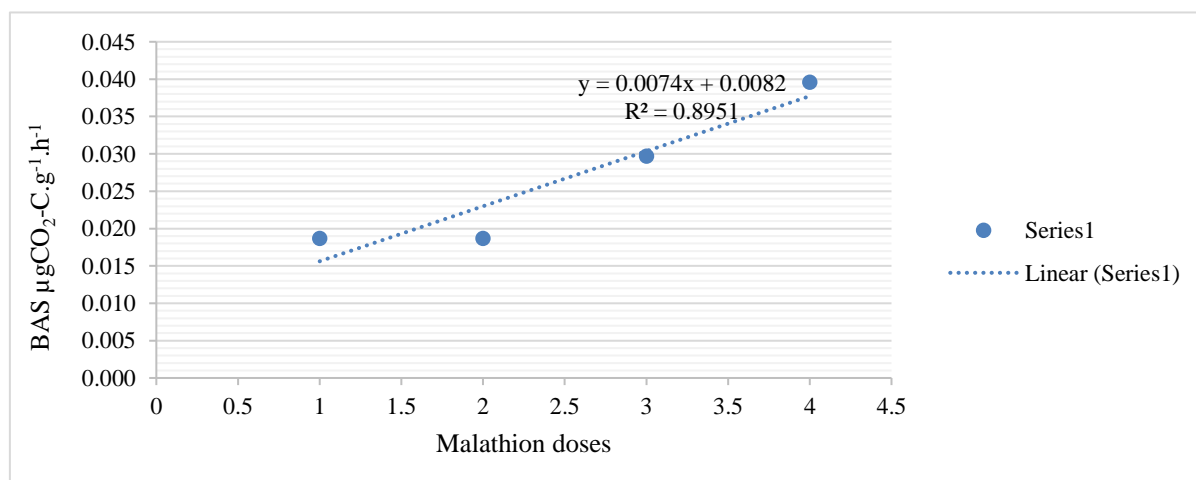


Figure 6: Correlation between malathion doses and basal respiration rate.

4. CONCLUSION

Repeated application of pesticides in soil cause various changes in soil quality parameters. During this study, we concluded that the increasing of malathion dose caused increasing in dehydrogenase activity and soil respiration rate after two week of application, however increasing carbaryl doses decreased dehydrogenase activity while increased soil bacteria and respiration rate, but 10-fold application of carbaryl caused decreasing in soil respiration rate. Moreover, a significant positive correlation was observed between dehydrogenase and soil respiration rate after malathion application.

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