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The Effect of Fertilisation using *Trichoderma harzianum* and Cow Manure on Releasing CO_2 in Two Soils with Different Textures in North of Iraq

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Abstract: A laboratory experiment was conducted using the completely randomised design (CRD) to estimate CO_2 released from the soil in one of the labs at the College of Agriculture and Forestry using two levels of fungus inoculant (without inoculation and inoculation with *Trichoderma harzianum*) with 10 cm^3, and two levels of cow amendment (without manuring and 2% manure). This experiment was conducted in two types of soils, the first was taken from one of the fields at the College of Agriculture and Forestry and the second soil was taken from the Zaweetah area, which is planted with pine trees targeting the release of CO_2 that is considered as an indicator of the biological decomposition of organic fertilisers. The experimental units were incubated for 10, 20, 40 and 60 days at a temperature of 28°C ±2. The results showed that inoculating the soil with *T. harzianum* fungus, in the presence of the organic amendment (cow manure), gave the highest value of CO_2 release at the incubation period of 40 days for both soils as the CO_2 release values were 52.9 and 57.7 mg.100 g^{-1} soil. Moreover, a decrease in the quantity of CO_2 released was observed at the incubation period of 60 days in both soils.

Key words: Organic fertilisers, biological decomposition, *Trichoderma harzianum*.

Introduction

Lime soils are known as soils that include certain quantities of calcium carbonate and are characterised by high quality surface areas due to their fine particles that negatively affect the availability of the soil to nutrients (Kadry, 1973). Also, Hagin and Tucker (1982) defined lime soil has the levels of extractable Ca and Mg, which are more than the cation exchange capacity. AlAmiri (2011) found that the organic matter decomposition, whether it is sheep or cow amendment, speed and CO_2 release increase are more in the lime soils compared to the gypsum soils.

In the meantime, the whole world is inclined to adapt clean agricultural technologies and minimise, as much as possible, the pollution hazards. For this reason, biological and organic fertilisers are used instead of chemical ones (El-Akabawy, 2000). Organic fertilisers are of great importance due to the nutrients they offer especially nitrogen (Ouda and AlEśa, 2003). Jabbar and AlŚheikhli (2013) found the high percentage of the aggregates by adding the biological and organic fertilisers. Also, Rośen and Bierman (2007) mentioned that the organic fertilisers help conserving the moisture content of the soil and the increase of organic amendment mineralisation in the soil causes a steady decrease in carbon to nitrogen ratio (Hartley and Ineśon, 2008). Walpola and Arunakumara (2010), AlObaidi and Mohammed (2009) and AlAmiri (2011) found that the speed of decomposition varies according to the ratio

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of the carbon to nitrogen of the organic amendment, as this speed increases with the decrease of the ratio of carbon to nitrogen.

Organic carbon is the main element of soil fertility as it enhances the structure, health and physical and biological qualities of the soil by means of attaching the particles of the soil and changing them into cohesive soils. AlObaidi and Ali (2009), Śalih and Ali (2017) and AlAmiri (2011) found that there was an increase in the quantity of CO_2 release with the increase of the organic fertiliser incubation in the soil, as well as its high ability to give the plant host high resistance against some pathogens as this contributes to the formation of nutrients in the soil through the release of CO_2 to the atmosphere.

The ratio of the organic carbon in the soil differs according to the type of the soil as storing an abundant quantity of carbon in the soil decreases the carbon content in the atmosphere and this limits the global warming phenomenon. Carbon contributes to the hydrogen changes that occur in the soil (Qaśim and Ali, 1989).

Biological fertilisers are regarded as one of the modern technologies used to mitigate the excessive use of chemical fertilisers. In recent years, these fertilisers have attracted much attention to be used as safe alternative methods to enhance the soil properties and its content of some elements like phosphorus and nitrogen (Ali, 2015). In addition to that, inoculation using fungi increases the content of N, P and K in the plants (Harbawe, 2014). In order to mitigate the soil pollution problems caused by chemical fertilisers, biological activity should be promoted by means of using biological inoculation techniques (Jabbar and AlŚheikhli, 2013). Moreover, Atrośhy (2004) and Janśśenś (2006) suggested that the metabolic activity of the thermophilic microbes increases the temperature.

The fungus *T. harzianum* is regarded as one of the fungi belonging to the class Saprophyte that is used in the biocontrol field on a wide scale as proven by several experiments in various countries around the world, especially in terms of increasing the availability of some elements such as nitrogen, phosphorus and potassium by secreting some enzymes and its high ability in providing the host plant with high resistance against some pathogens (AlHadeethi, 2002; Altomare et al., 1999; Harman, 2000).

The study aims at investigating the role of *Tricoderma harzianum* fungus and its importance in decomposing the organic matter and the release of CO_2 in the lime soils, thereby enhancing the physical properties of the soil and nutrients availability.

Materials and Methods

Collecting the Soils and Preparing them for Planting

In this study, two soil samples were taken from different depths (0 and 30 cm) and different locations, the first sample was taken from an unplanted field affiliated with the College of Agriculture and Forestry at Mosul University. Then the soil was mixed and blended to give a compound sample that could represent the field. The second sample was taken from the Zaweetah area in Duhok Governorate from a location planted with pine trees. The sample was taken to the laboratory and dried under air, ground and filtered through a 2mm sieve to conduct the routine analyses, as shown in Table 1.

Preparation of the Organic Amendment

Partially fermented cow manure was used as an organic source. It was dried by air, ground and filtered through a 2 mm sieve to conduct the chemical analyses. Table 1 shows some of the chemical properties of the cow manure (AlTaee, 2013).

Preparation of the Spore Suspension

A plate that contains an isolate of *T. harzianum* was taken from the Nematode laboratory that belongs to Plant Protection Department. The fungal isolate was inoculated on the solid Martin Media in 250 cm^3 conical flasks and then put in an incubator for one week at a temperature of 28°C ±2. After the completion of growth, 10 cm^3 of sterilised water was added and then the spores were scattered on the surface of the environment with the help of water by inoculation needle to convey the spores to the water so that a dense spore suspension can be formed and to bring the number of transactions to 64 transactions.

An experiment was conducted with three factors, such as the organic matter, inoculation agent and the period of incubation with three replicates within the completely randomised design (CRD), by taking 100 grams of the soil and treating it with the organic matter (cow manure) and compared to the (control) treatment (without adding cow amendment). The addition was performed on the basis of the ratio of organic carbon in the soil. The cow manure was added so that the organic carbon percentage becomes 2%. The soil was put in a sealed plastic bottle and water was added to the moisture content equivalent to 75% of the field capacity, and the

Table 1: Physical, chemical and biological properties of the two soils taken for the study

Property		*Unit*	*Measurement*		
			Soil from the College of Agriculture and Forestry	*Zaweetah soil*	*Cow manure*
Soil separations	Sand	$g.kg^{-1}$	299.50	251	
	Clay		269.00	420	
	Celt		431.50	329	
Texture			Celt clay	Clay Lime	
Field capacity		$g.kg^{-1}$	20	27.5	
Exchange capacity of cations		$Cent.mole.kg^{-1}$	22.83	0.70	
Electrical conductivity		$dS.m^{-1}$	0.62	7.37	8.34
pH of soil reaction (1:1)			7.6	32.3	8.21
Organic matter		$g.kg^{-1}$	8.03	310	
Calcium carbonate			350		
Organic carbon					286
Content of available elements	Nitrogen	$g.kg^{-1}$	31.00	19	
	Phosphorus		10	6.7	
	Potassium		320	200	

lost water was compensated by recording the weights of the bottles at the required moisture by adding water. The samples were incubated at a temperature of 28°C ±2 in incubation periods of 10, 20, 40 and 60 days.

Figure 1 shows the treatments used in the experiment.

The quantity of CO_2 was estimated for the incubation periods 10, 20, 40 and 60 days according to the Stotzoky method mentioned by Black (1965b). A small beaker, which includes 10 ml of sodium hydroxide (1N) was placed inside the sealed bottles to prevent the loss of the CO_2 released, because the sodium hydroxide solution reacts with CO_2 present in the air which is now trapped in the bottle and the result will be the turbid sodium carbonate that is precipitated by adding 5 ml of barium chloride (1N) as in the following equations:

$$2NaOH + CO_2 \longrightarrow NaCO_3$$

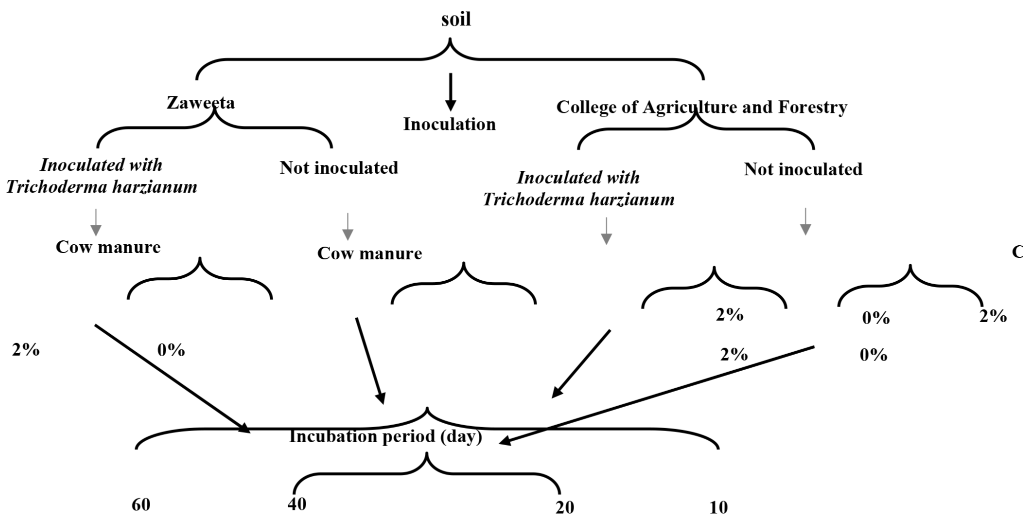

Figure 1: Diagram of transactions in the experiment.

Turbid solution

$NaCO_3 + BaCl_2 \longrightarrow 2NaCl + BaCO_3$ (white precipitate)

Then the samples were titrated with hydrochloric acid (0.5N) with the presence of the phenolphthalein reagent as an indicator to show the interaction end point, and the quantity of CO_2 was measured using the following equation:

$$\text{mg } CO_2 / 100 \text{ gm soil} = (B - V)\, NE$$

where, B = the volume of the acid consumed (ml) in the comparison. V = the volume of the acid consumed (ml) in the treatment. N = normality of Hydrochloric acid (HCl). E = the equivalent weight of (CO_2), which equals 22.

Results were statistically analysed using the SPSS package.

Results and Discussion

The results given in Tables 2 and 3 indicate the superiority of the treatment inoculated with *T. harzianum* fungus and fertilised with cow amendment at the incubation period (40 days) over the rest of the treatments as the values were 52.90 mg CO_2.100 g^{-1} for the soil collected from the College of Agriculture and Forestry. While the soil collected from Zaweetah area showed values of 48.1 and 57.46 mg CO_2.100 g^{-1} soil. This is in conformity with the study by Silva Junior et al. (2009), which showed that adding the organic matter increases the quantity of soil, eventually mineralising to carbon. On the other hand, the treatment inoculated with *T. harzianum* and fertilised with cow amendment was superior and gave the highest values compared to the treatment that is not inoculated for both soils as the values were (57.46 and 52.90) mg Co_2.g^{-1} soil respectively, and this may be due to the activity of the microbes that decompose the organic matter and lead to the release of CO_2. This is in conformity with the findings by AlRawi (2000) and Omran (2005), as they argued that the increase in the activity of microbes results in the decomposition of cow amendment and consequently the release of CO_2. In addition to that, the periods of incubation had a significant effect on the release of CO_2 and the availability of the minor elements with the suitable temperature and moisture content (AlAmiri, 2011). Also, the increase in the quantity of CO_2 released is due to the quantity of the

Table 2: The effect of inoculation with *Trichoderma harzianum* fungus and fertilising with cow amendments on releasing CO_2 in the soil of the College of Agriculture and Forestry

Inoculation	*Fertilising with cow manure*	*Period of incubation (day)*				*Inoculation effect × fertilisation*	*Inoculation effect*	*Fertilisation effect*
		10	*20*	*40*	*60*			
Zero	Zero	15.46 O	21.53 L	23.96 J	17.03 N	19.50 D		
	2%	19.36M	24.03 J	33.26 C	25.46 I	25.52 C		
T. harzianum	Zero	22.63 K	33.60 E	47.96 F	29.20 G	33.34 B		
	2%	27.50 H	49.13 B	52.90 A	43.93 D	43.36 A		
Interference between inoculation and incubation period	Zero	17.41 H	22.78 F	28.61 D	21.25 G		22.51 B	
	T. harzianum	25.06 E	41.36 B	50.43 A	36.56 C		38.35 A	
Interference between fertilisation and incubation period	Zero	19.05 H	27.56 E	35.96 C	23.11 G			26.42 B
	2%	23.43 F	36.96 B	43.08 A	34.70 D			34.45 A
Effect of the incubation period		21.24 D	32.07 B	39.52 A	28.90 C			

Table 3: The effect of inoculation with *Trichoderma harzianum* fungus and fertilising with cow amendments on releasing CO_2 in Zaweetah soil

Inoculation	*Fertilising with cow manure*	*Period of incubation (day)*				*Inoculation effect × fertilisation*	*Inoculation effect*	*Fertilisation effect*
		10	*20*	*40*	*60*			
Zero	Zero	18.36M	24.8 H	25.06H	24.56 I	23.20 D		
	2%	21.66 L	26.13G	33.60E	26.26 J	26.91 C		
T. harzianum	Zero	22.76 K	36.66D	52.13B	23.8 G	33.84 B		
	2%	28.43 F	52.36B	57.46A	41.36C	44.90 A		
Interference between inoculation and incubation period	Zero	20.01 F	25.46E	29.33D	25.41 E		25.05 B	
	T. harzianum	25.60 E	44.51 B	54.80 A	32.58 C		39.37 A	
Interference between fertilisation and incubation period	Zero	20.56 H	30.73 E	38.60 C	24.18 G			28.52 B
	2%	25.05 F	39.25 B	45.53 A	33.81 D			35.91 A
Effect of incubation period		22.80 D	34.99 B	42.06 A	29.00 C			

organic carbon, which results from the total nitrogen and the decrease of the ratio of carbon to nitrogen which is decomposed by the microbes. These findings conform with the results of Janzen and Kucey (1988), AlObaidi and Mohammed (2009) and Walpola and Arunkumara (2010).

The increase in the release of CO_2 quantities resulted due to the increase of the total content of nitrogen that is decomposed by the microbes, also there was an increase in the quantity of volatile CO_2 with the increase of time period, during which the organic fertiliser remains in the soil. On the other hand, the CO_2 quantity decreased at the incubation period of 60 days and this decrease may be due to the decrease of the organic matter quantity with time as it is consumed by the soil microbes and eventually the quantity of CO_2 in the soil decreases. This was also indicated by Jarallah (2014) who mentioned that there is an increase in the quantity of CO_2 with the presence of cow amendment at the incubation period of 50 days, and then this value decreases at the period of 70 days. This decrease could be due to low activity of the microbes and low use of the organic matter by them and this corresponds with what Raśul et al. (2006) found, which means that the direct use of the organic matter leads to maintain the metabolic activity of the microbes.

Conclusion

Results showed that inoculation of the soil samples with *T. harzianum* fungus, in the presence of the organic amendment (cow manure), gave the highest value of CO_2 release at the incubation period of 40 days for both soils as the CO_2 release values were 52.9 and 57.7 mg.100 g^{-1}, respectively. Moreover, a decrease in the quantity of CO_2 released was observed at the incubation period of 60 days in both soils.

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