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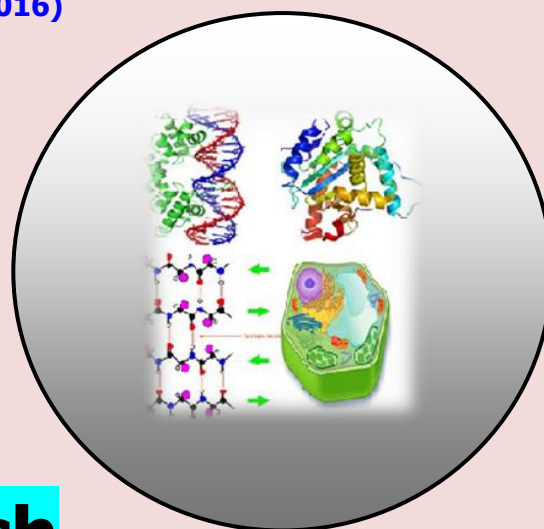
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Effects of Fungicides, Incubation Time and Temperature on Soil Respiration in Laboratory Conditions**Çiğdem Küçük**

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ABSTRACT

The effects of mancozeb and carbendazim on soil respiration were determined through a 30 day incubation period at 20 oC, 25 oC and 30 oC. Soil sampling was carried out after 5, 10, 15, 20, 25 and 30th days of incubation. Soil respiration showed fluctuation with kind of the fungicide. Soil respiration was significantly ($p<0.001$) affected from the fungicides, temperature x fungicide, temperature x incubation time and incubation time. In this study, when carbendazim was added to soil, soil respiration increased at 20 oC. At 30 oC, soil respiratory increased that at the 10 th day when the soil was added to mancozeb and at the 20 th day of incubation period when it was added with carbendazim. It was concluded that fungicide and temperature applications affect soil respiration.

Keywords : Soil, Respiration and Temperature.

INTRODUCTION

Chemical fertilizers and pesticides has unwanted side effects (Kalia et al. 2011). Fungicides are biotoxicants that not only interfere with the target pathogenic microorganisms but can also affect the non-target microorganisms in the soil (Bending et al. 2006; Lo 2010). Application or widespread use of pesticides in the soil affects the quality of the organic matter in the soil and the diversity of the microbial population (Lo 2010). In general, the effects of fungicides on soil are vary depending on the used doses of fungicides, environment conditions (Munier-Lamy and Borrde 2000).

Agrochemicals applied in the soil for long periods have negative impacts on soil microbial population (Araujo et al. 2003). When fungicides are applied in soil, they may alter the equilibrium of soil biological processes. Soil microorganisms play a vital role in many soil biological process (Casida 2009). Fungicides have been target various pathways that disrupt basic cellular functions, protein biosynthesis i.e. tubulin biosynthesis or essential enzymes (Casida 2009).

The soil microbiological properties are the sensitive to fungicides. Fungicides effects on biological processes. In recent years, several reports have reported the harmful effects on soil microorganisms and soil microbiological activity of fungicides in soil (Cernohlavkova et al. 2009; Munoz-Leoz et al. 2011; Yunlong et al. 2009; Xiong et al. 2013). Cernohlavkova et al. (2009) have studied that effect of mancozeb and dinocap on carbon and nitrogen mineralization in soils in laboratory conditions. Xiong et al. (2013) have tested that effect of pynimorph on soil enzyme activities and soil respiration. Magarey and Bull (2003) have reported that decreased number of fungi and increased number of total bacteria exposed to mancozeb. It is known that soil respiration is influenced by a number of complex factors such as temperature, humidity, soil properties, soil organic matter quality, quantity and fragmentation characteristics (Kirschbaum 1995; Raich and Tüfekçioğlu 2000). CO₂, which is the result of the activities of microorganisms, is regarded as a sign of microbial activity in the soil (Raich and Tüfekçioğlu 2000). In Turkey, pesticides are consumed against various plant diseases, and

the use of carbamic acid, benzimidazole group fungicides and pesticides has increased in recent years. As a result, environmental pollution has arisen due to accumulation in water, air, soil and foodstuffs of applied fungicides (Ahtianenet al. 2003).

Fungicides applied especially for vegetable, potato, cotton, fruit production. Mancozeb is highly toxic to soil microorganisms, fish and aquatic organisms (Lo 2010). Carbendazim and mancozeb are commonly recommended for disease control in Turkey. The aim of this study is to evaluate the effects of mancozeb and carbendazim on soil respiration at different temperature conditions.

MATERIALS AND METHODS

Sampling and analysis

The soil pH was measured in a 1:2.5 soil mixture (soil:water) with a pH meter, soil organic matter content was determined by a modified Walkley Method (1964), the soil particle size distribution was determined by the Bouyoucours hydrometer (1951) and the total N content by Kjeldahl method (Bremner1982). An incubation experiment was conducted in pots, each containing 250 g sieved soil with various treatments. Fungicides were added into soil samples. Soil concentrations of mancozeb and carbendazim were applied at approximately field application rates (200 g 100 l⁻¹ for mancozeb and carbendazim). In addition, the soil samples were watered at 60 % of the field capacity. After the soils were incubated at different temperature (20 °C, 25 °C and 30 °C) and 5, 10, 15, 20, 25 and 30 days. Soil samples analyzed for soil respiration. Each treatment was replicated three times and the experiment was carried out in a split-plot. Temperature treatments were established as main plots and arranged in a randomized complete block design. Fungicides were randomized as subplots.

Basal respiration

Basal soil respiration was determined by the titrimetric method (Anderson 1982). The moist soil was placed in an airtight jar containing a vial with Ba(OH)₂ and incubated at 28°C. After days of incubation, the Ba(OH)₂ solution vial was removed from the jar, BaCl₂ and phenolphthalein added and was titrated out with HCl. Soil respiration as CO₂-C was computed from the titration value according to the following equation of Anderson(1982).

Statistical analysis

LS Means Differences Student's t was used for comparison of the mean values at the 1% level of significance using software JMP11.

RESULTS AND DISCUSSION

The pH of the soil was 7.74, particle ratio was 53.12:24:22.8 % (clay:silt:sand %, respectively) and the soil organic matter content was 1.71 % (Table 1). Electrical Conductivity (EC) was 2.45 mmhos/cm, total N content 0.18 %, available K₂O content 97.2 kg/da. The soil can be classified as unpolluted. The effects of two fungicides (Mancozeb and Carbendazim), widely used in agriculture were shown in Table 1.

Basal respiration is commonly used parameter reflecting microbial activity, which allows assessing the potential perturbations of the carbon transformation processes in soil treated with fungicides and a xenobiotics (Domsch et al. 1983). Cernohlavkova et al.(2009) observed that mancozeb increased soil respiration. Changes in soil respiration treated with fungicides during incubation are shown in Figure 1-3. The higher the amount of carbon dioxide in the pots applied to the mancozeb. Mancozeb is thought to promote the growth of the microbial population on 10 th day at 25°C and on 20th day at 20°C.

Digrak *et al.* (1996) reported that the application of mancozeb was increased microbial populations in the soil. The results of the researchers supported our work. In the soil amended with carbendazim had different effect on respiration (Fig.1). In mancozeb and carbendazim with applications, basal respiration was stimulated according to the control. However, increases in soil respiration could result from the degradation of fungicides used as a substrate by soil microorganisms. The strong stimulation of respiration was also found by Cernohlavkova et al. (2009) and Xiong et al. (2013). Some fungicides degrading from microorganisms and the those using other ingredients of the preparation may be responsible for increased respiration ascertained. The changes in microbial respiration in soil amended with fungicides e.g. captan, tebuconazole, benomyl have been related closely to the dosages of pesticides used (Pozo et al. 1994). Our results are agreement with results of Cycon et al.(2006), Martinez-Toledo et al. (1998) and Mercadier et al. (1997).

At 20 °C; the higher respiration rate in the soil treated with carbendazim on 10th day compared to the control, suggests that it is caused by the stimulating effect of carbendazim on the soil microorganisms.

The structure and concentrations of chemicals are found to be effective on microorganisms as well as that the effect of fungicides on microorganisms in the soil depends on the environmental conditions such as pH, light and temperature (Ahtiainen et al.2003). The amount of carbon dioxide in the soil where fungicides were applied varied during the incubation period (Table 2).

Table 1. Some physical and chemical properties of soil.

Characteristics	Value
Particle size distribution	
Sand (%)	22.88
Silt (%)	24
Clay (%)	53.12
Texture grade	clay
pH	7.66
EC (dS m ⁻¹ at 25 °C)	1.65
Total N (%)	0.18
Available K (kg/da)	97.2
CaCO ₃	20.8
Organic matter (%)	1.71

Table 2. Effects on soil respiration of temperature, incubation time and fungicides.

Temperature	Incubation time (day)	Treatment		
		Control	Carbendazim	Mancozeb
20 °C	5	30.6 no	20 m	31.8 no
	10	39.7 hi	75.5ef	17.9 wz
	15	11 wz	416 b	35.2 st
	20	19.8 w	39.3 ij	43.7 kl
	25	13.5 z	24.3 pq	26.5 m
	30	8.9 wz	20.9 wz	15.9 rs
25 °C	5	32.7 w	18 x	46.7 w
	10	43 a	26.1 stu	52.3 qr
	15	57.2 fg	36.9 jk	35.5 no
	20	38.3 l	39.4 l	39.5 gh
	25	28.6 v	13.9 z	26.3 m
	30	6.9 w	4.6 wz	30.5 tuv
30 °C	5	31.8 no	46.7 w	30 op
	10	17.9 wz	52.3 qr	44.3 e
	15	35.2 st	35.5 no	24.7uv
	20	43.7 kl	39.5 gh	36.7 kl
	25	26.5 m	26.3 m	35.7 kl
	30	15.9 rs	30.5 tuv	17.4 x

Table 3. Analysis of variation of treatments.

Source	df	F ratio	Prob>F
Temperature (T)	2	1.90	<.0001
Fungicide (F)	2	408.63	<.0001
Incubation time (I)	5	1948.59	<.0001
T x F	4	1396.63	<.0001
T x I	10	485.96	<.0001
F x I	10	215.85	<.0001
T x F x I	20	538.9	<.0001

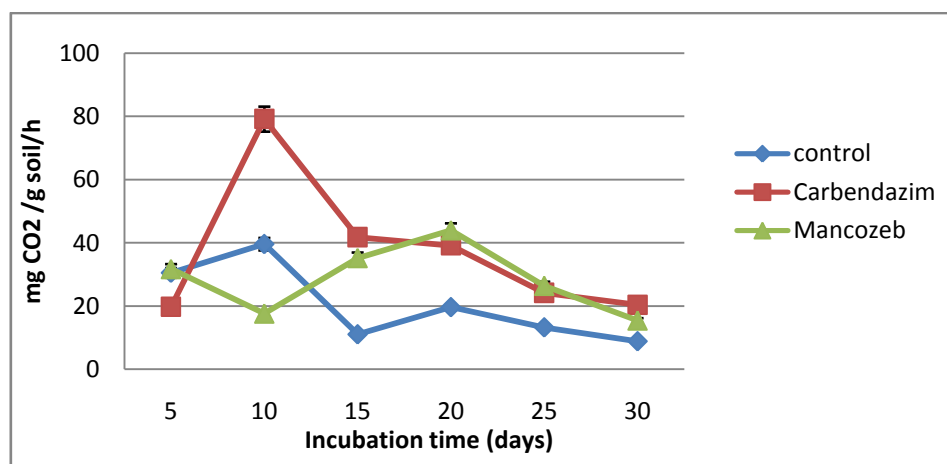


Figure 1. Effects on soil respiration of carbendazim and mancozeb on different incubation days at 20 °C.

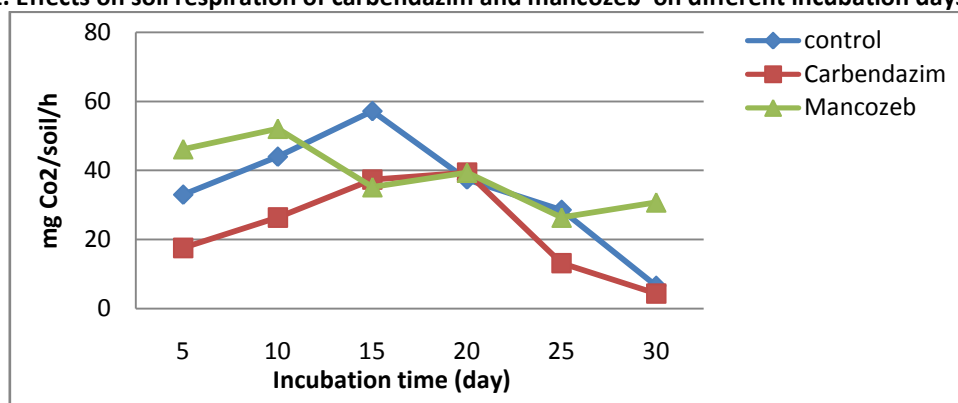


Figure 2. Effects on soil respiration of carbendazim and mancozeb on different incubation days at 25 °C

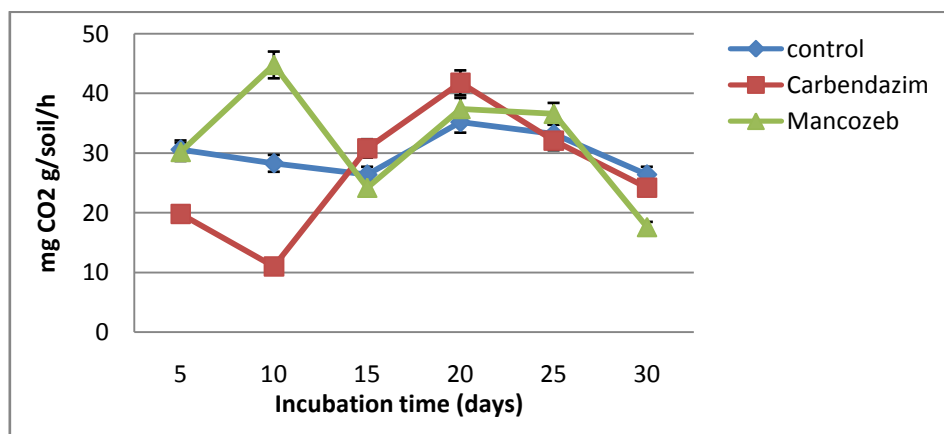


Figure 3. Effects on soil respiration of carbendazim and mancozeb on different incubation days at 30 °C

During the incubation period, the CO₂ content in the control pots varied at all three incubation temperatures. The highest amount of carbon dioxide was taken at 10 °C and at 20 °C at carbendazim application. This increase may be considered to be the use of fungicide as a source of carbon and energy in some microorganisms. Also, the increase in microbial populations in soils also affects the rate of carbon dioxide.

In this study showed that the fluctuation at respiration was observed induring the incubation period (Fig. 1-3). The variance analysis of the obtained data, showed that the respiration had significantly affects on temperature, fungicide, temperature x fungicide, temperature x incubation time, incubation time, fungicide x incubation time and temperature x fungicide x incubation time (Table 3).

In the previous study reported that, soil respiration has been obtained to be fluctuation after application of fungicides to the soil (Domsch et al. 1983). Such phenomena were probably based on the increased populations and activity of a few resistant microorganism or were due to direct microbial utilization of fungicides as substrates (Cycon et al. 2006; Vyas 1988). On the other hand, Pozo et al. (1994) reported that total fungal populations, denitrifying bacteria and aerobic bacteria population significantly decreased by mancozeb while total bacteria were not affected by the addition of mancozeb. Also, Pozo et al. (1994) concluded that some microbial groups can tolerate used doses of mancozeb.

CONCLUSION

We have found that two fungicides commonly used in agriculture were affected soil respiration within the short incubation period (30 day). The effects of the fungicides on soil respiration were specific to each fungicide. Therefore, soil respiration as a result of metabolic activity of complex soil microorganisms was affected from fungicides treated soils. Microbial activity in soil is frequently restricted by the level of available nutrients. A proportion of this activity in the soil can be stimulated by the addition of substrates.

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