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CHANGES IN SOIL CARBON MINERALIZATION UNDER THE EFFECTS OF FUNGICIDE CYPRODINIL

B. Koçak

Department of Biology, Faculty of Arts and Sciences, Cukurova University, Adana, Turkey

*Corresponding author: bkocak@cu.edu.tr

Abstract

Soil organic carbon mineralization refers to the overall processes by which microorganisms mineralize soil organic C and release it in the form of CO_2. A short term experiment was carried out to ascertain the effects of different recommended doses (RD) of fungicide cyprodinil [0 (Control), 10.7 (RD), 21.4 (RD x2) and 42.9 mg/ kg soil (RD x4)] on soil carbon mineralization using an agricultural soil. Soil was sampled from Research Farm of Faculty of Agriculture in Cukurova University (Adana, Turkey) in June 2018. This soil had no history of cyprodinil usage. After the determination of some soil physical and chemical properties, soils were humidified at 80% of field capacity, mixed with doses of the fungicide tebuconazole and incubated for 42 days at 28°C. CO_2 respiration was measured during the following days of incubation: 1, 3, 6, 9, 12, 16, 23, 30, 37 and 42. Cumulative carbon mineralizations (mg $C(CO_2)$/ 100 g soil) were in the range between 68.56 (Control) and 79.20 (RD x4) while their rates were in between 3.78% (Control) and 4.37% (RD x4) at the end of incubation period. All doses of cyprodinil significantly increased carbon mineralization ($P<0.05$). Stimulations of microbial activity by doses of fungicide cyprodinil were 9.9% for RD, 10.5% for RD x2 and 15.5% for RD x4. It's suggested that microorganisms in this soil may positively mineralize both recommended field dose of fungicide cyprodinil and its higher doses.

Keywords: Fungicide, Aminopyrimidine, Cyprodinil, Soil microbial activity, Soil carbon mineralization

INTRODUCTION

Pesticides are the important chemical compounds that are being used in agricultural systems for protection of crops from pests including weeds, insects etc. Pesticides are usually applied for many times during one crop season and a part of these chemicals always enter to the soil. Many problems including the pollution of the environment have been created by the wide usage of pesticides (Arora and Sahni, 2016). The effects of pesticides on soil microorganisms are depend on physical, chemical and biochemical conditions, in addition to nature and concentration levels of the pesticides (Aurelia, 2009; Sethi et al., 2013). In many studies, it was shown that microorganisms are capable to grow and maintain their lives in the presence of several commercial pesticides. If a soil microorganism can use the pesticide as a carbon and energy source, then catabolism and detoxification metabolism may occur. Most pesticides work by poisoning pests (Arora and Sahni, 2016).In general, a systemic pesticide moves inside a plant following the absorption of plant. This movement is often upward (through xylem) and outward with most fungicides (Shinde et al., 2015)

Cyprodinil was first applied on cereals in France in 1993. This fungicide has been used on cereals, vegetables, fruits, field crops and ornamental plants as foliar application (Waechter et al., 2010). Products contain cyprodinil were marketed in most European countries, in North America and in Japan. Cyprodinil was also used in control of pathogen fungi like *Pseudocercosporella herpotrichoides*, *Erysiphe* spp., *Pyrenophora teres*, *Rhynchosporium secalis*, *Septoria nodorum*, *Botrytis* spp., *Alternaria* spp., *Venturia* spp., and *Monilinia* spp. (Waechter et al., 2010).

Formulations used in foliar applications were as WG (water dispersible molecules) or EC (emulsifiable concentrates) type.

Cyprodinil was identified as a inhibitor of methionine biosynthesis in liquid cultures of *Botrytis cinerea*. It was reported that pathway of cystathionine in filamentous fungi like *Neurospora grassa* and *Aspergillus nidulans* has been established as the the major route of homocysteine and methionine biosynthesis (Yamagata, 1989). In this pathway, cyprodinil targets the cystathionine β-lyase that synthesize cystathionine from homocysteine (Fritz et al., 1997; Masner et al., 1994). Secondary target of cyprodinil was the secretion system of plant cell wall degrading enzymes in pathogenic fungi. It is believed that these enzymes have an important role in the early periods of plant infection and penetration of plant tissues by fungi mycelium was inhibited in the absence of secretion of these enzymes (Milling & Richardson, 1995; Miura et al., 1994).

Soil microbial activities are critical in decomposition of processes (Cernohlavkova et al., 2009). However, intensive applications of chemicals used in agriculture negatively influence on these processes in many soils. Stability and productivity of soil ecosystems depend largely on soil microorganisms and their activity. For this reason, determination of side effects of pesticides on soil microbial communities is very critical for soil health. Carbon mineralization is an important parameter that adopted in international guidelines for ecological risk assessment and standard tests of pesticides and other chemicals (OECD, 2000). After pesticide applications, there were several studies that soil carbon mineralization was increased in field and laboratory experiments (Das & Mukherjee, 2000; Monkiedje et al., 2002). Carbon mineralization (Cmin) is an age-old reliable method for studying microbial activities in soil. Changes in carbon mineralization have also been used as criteria for pesticide toxicity (Torstensson & Stenström, 1986).

The objective of this study was to evaluate the effects of cyprodinil on soil C mineralization. Recommended field dose (as it was written its etiquette, RD) and its 2 (RD x2) and 4 (RD x4) folds of cyprodinil was mixed with an agricultural soil and CO_2 respirations were measured at 28°C for 42 days. It was hypothesized that fungicide cyprodinil would increase soil carbon mineralization.

MATERIALS AND METHODS

Soil Characterization

Soil samples that were not previously exposed to fungicide cyprodinil were collected from the top layer (0-10 cm) in Research Farm of Faculty of Agriculture in Cukurova University in Adana, Turkey (35°18′ E latitude, 37° 01′ N longitude, and 23 m above sea level) in June 2018. Immediately after collection, soil samples were taken to the laboratory in dark plastic bags, homogenized, air-dried at 25°C during 96 h, sieved to 2 mm to remove plant tissue and subjected to physicochemical characterization.

Soil texture was determined with a Bouyoucos hydrometer. Soil pH was measured in mud saturated with distilled water (1:2.5) using a WTW Inolab 720 pH meter. The $CaCO_3$ contents of the soils were estimated with a Scheibler calcimeter. The organic carbon and total nitrogen contents of the samples (%) were determined with the modified Walkley-Black and Kjeldahl methods, respectively (Kacar, 2009). The field capacity (FC) of soils (%) were determined with a vacuum pump with 1/3 atmospheric pressure (Kacar, 2009).

Incubation Experiment

Soil samples (100 g) were placed in 750 ml incubation vessels and mixed with and without fungicide cyprodinil (300 EC) at the recommended field dose and its 2 and 4 folds [0 (Control), 10.7 (RD), 21.4 (RD x2) and 42.9 mg/ kg soil (RD x4)]. The amount used in RD treatment was the recommended field rate for cyprodinil (300 g active ingredient/l) that was written its etiquette with the assumption that the soil weight was 1.4 x 106 g/da at the effective soil depth of 1 mm. Three replicates were used for each treatments and control. Empty vessels were used as blanks. The final moisture contents of both soils were adjusted to 80% of their own field capacity before incubation at 28°C for 42 days. Throughout the incubation period, deionized water was added to the soil to compensate for any water loss. CO_2 derived from microbial activities was absorbed in 10 ml 1 M NaOH solution in bechers, placed in the center of the soils in closed incubation vessels, and then

transferred to a dark incubator. The amount of CO_2 produced was measured by titration with hydrochloric acid in the following days of incubation: 1, 3, 6, 9, 12, 16, 23, 30, 37, 42 (Alef, 1995). Cumulative carbon mineralization (mg C(CO_2)/100 g soil) was calculated by summing up all CO_2 measured days until end of incubation period while its rate was calculated by dividing cumulative mineralized C by its soil organic C of control and all applications for 42 days.

Statistical Analysis

Tukey honestly significant difference (HSD) in analysis of variance (ANOVA) was performed to determine the differences between treatments in soil carbon mineralization (Kleinbaum, 1998). All of the tests were performed at the significance level of $P<0.05$. Statistical analysis was carried out using the SPSS v20**.**

RESULTS AND DISCUSSION

Some soil physical and chemical properties of soil sampled from Research Farm of Cukurova University were given in Table 1. Soil was classified as loam and slightly basic. Soil organic carbon and total nitrogen were 1.81 (%) and 0.163 (%), respectively (Table 1). Soil carbon/nitrogen ratio was 11.20 (Table 1).

Table 1. Some physical and chemical properties of soil

Soil Characteristics	
Sand (%)	41,90 ± 0,06
Silt (%)	32,90 ± 0,06
Clay (%)	25,20 ± 0,10
Texture	Loam
Field Capacity (%)	24,52 ± 0,06
pH	7,89 ± 0,03
$CaCO_3$ (%)	41,80 ± 0,84
C (%)	1,81 ± 0,01
N (%)	0,163 ± 0,008
C/N	11,20 ± 0,55

The effects of fungicides on microbial processes and soil microorganisms depend on many factors. Some of major factors include physical, chemical and biological properties (such as organic matter, pH, temperature and humidity) of soil and nature and dose of the fungicides applying and its time (Vyas, 1988; Chen et al., 2001).

Carbon mineralization is an important parameter for determining the side effects of pesticides (Sommerville, 1987; Alef, 1995). Soil carbon mineralizations (mg C(CO_2)/100 g soil) were in the range between 3.92 and 10.18 for control, between 4.58 and 10.18 for RD, between 3.66 and 10.99 for RD x2 and between 1.22 and 11.20 for RD x4 (Figs. 1-2). In general, all doses of cyprodinil increased carbon mineralization in whole experiment. Significant differences between control and RD were found on 6.day and 37.day ($P<0.05$) and this data were similar between control and RD x2 (Fig.1 and Fig.2). No significant differences were found between RD and RD x2 except on 42.day. Carbon mineralization under RD x4 dose were significantly higher than control in all measured days except on 12.day and 42.day ($P<0.05$, Figs 1-2).

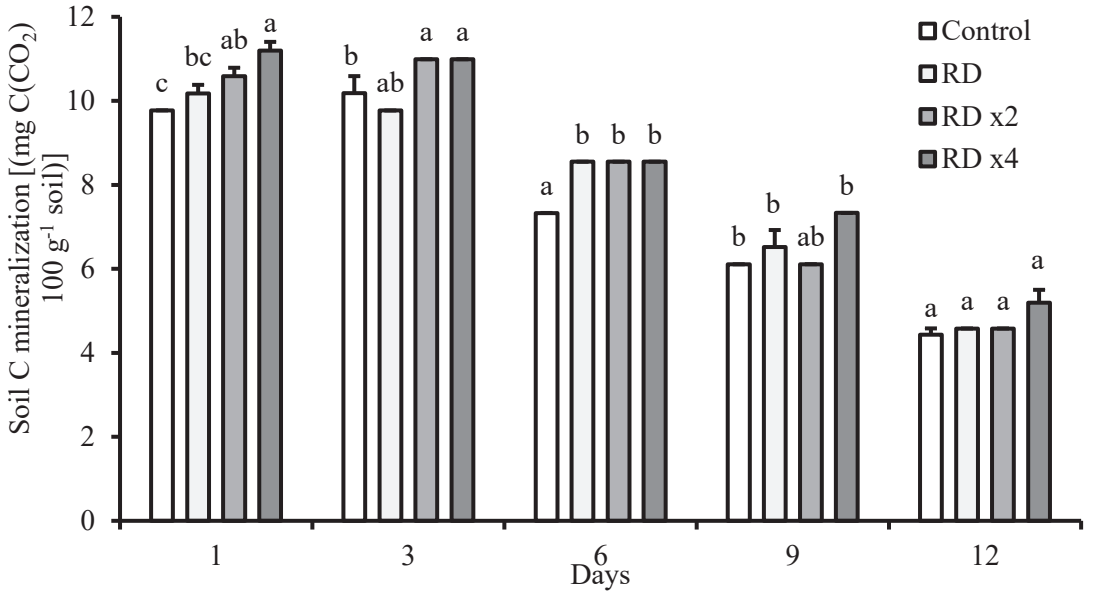


Figure 1. Soil carbon mineralizations between 1.day and 12.day at 28°C [mean± standard error, n=3, a, b and c indicate significant differences between means (P<0.05)]

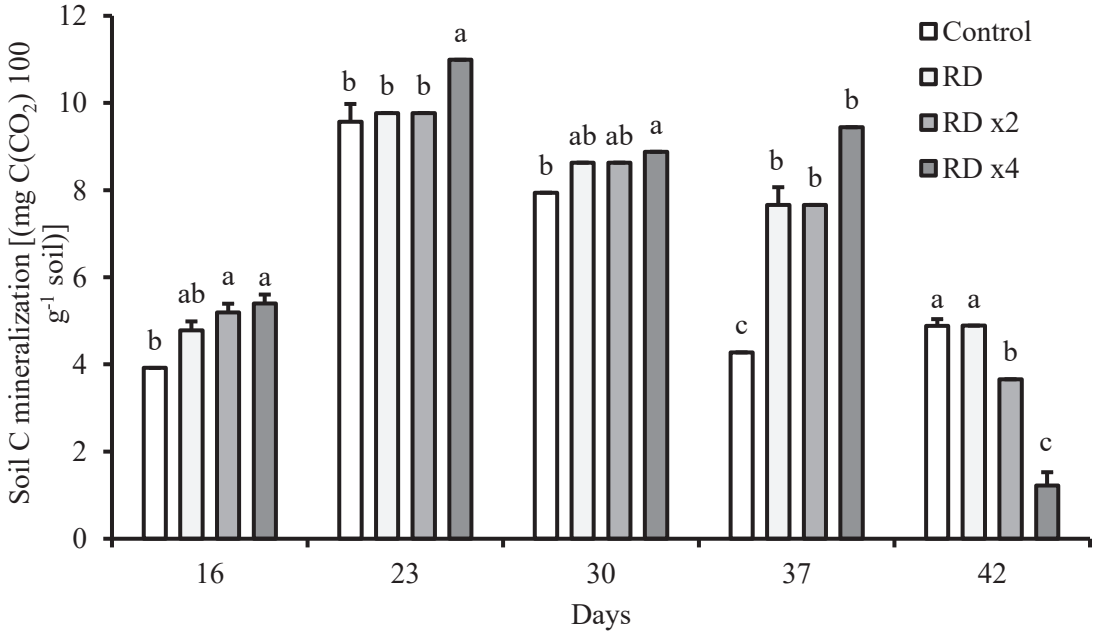


Figure 2. Soil carbon mineralizations between 16.day and 42.day at 28°C [mean± standard error, n=3, a, b and c indicate significant differences between means (P<0.05)]

Liu et al. (2011) compared the half-lives of cyprodinil (50% WG) in soil under field and greenhouse conditions at the dosage of 1,080 g active ingredient ha^{-1} and reported that pesticide cyprodinil degrades faster under greenhouse condition (6.5 days) than field condition (12.5 days) either in soil. Same authors indicated that the temperature and humidity were at almost fixed level (temperature of 25–30°C and humidity of 70–80% according to the experimental record) under the greenhouse condition. These results corralete within our research data. These experiments indicated that cyprodinil may be used as an energy source for soil microorganisms.

The previous studies about cyprodinil were mainly focused on its residues analysis, bound residue formation, photo-degradation in aqueous solution, and biosynthesis of reference standards by microorganisms (Munitz et al., 2013, Dec et al., 1997, Kang et al., 2002, Fenoll et al., 2011,

Chen et al., 2016). Although biodegradation of cyprodinil has been reported by Schocken et al. (Schocken et al., 1997), almost no literature is available about the effects of cyprodinil on soil carbon mineralization. Results of the current study indicated that recommended field dose and its 2 and 4 folds of cyprodinil enhanced ratio of C mineralization in this agricultural soil.

Cumulative carbon mineralizations (mg $C(CO_2)$/100 g soil) were in the range between 68.56 (Control) and 79.20 (RD x4) at the end of incubation period (Fig. 3). All doses of cyprodinil significantly increased soil carbon mineralizations compared to control ($P<0.05$). In contrast, there were found no significant differences between RD and RD x2. Increased percentage of soil carbon mineralizations were 9.9% for RD, 10.5% for RD x2 and 15.5 for RD x4 compared to control (Fig. 3).

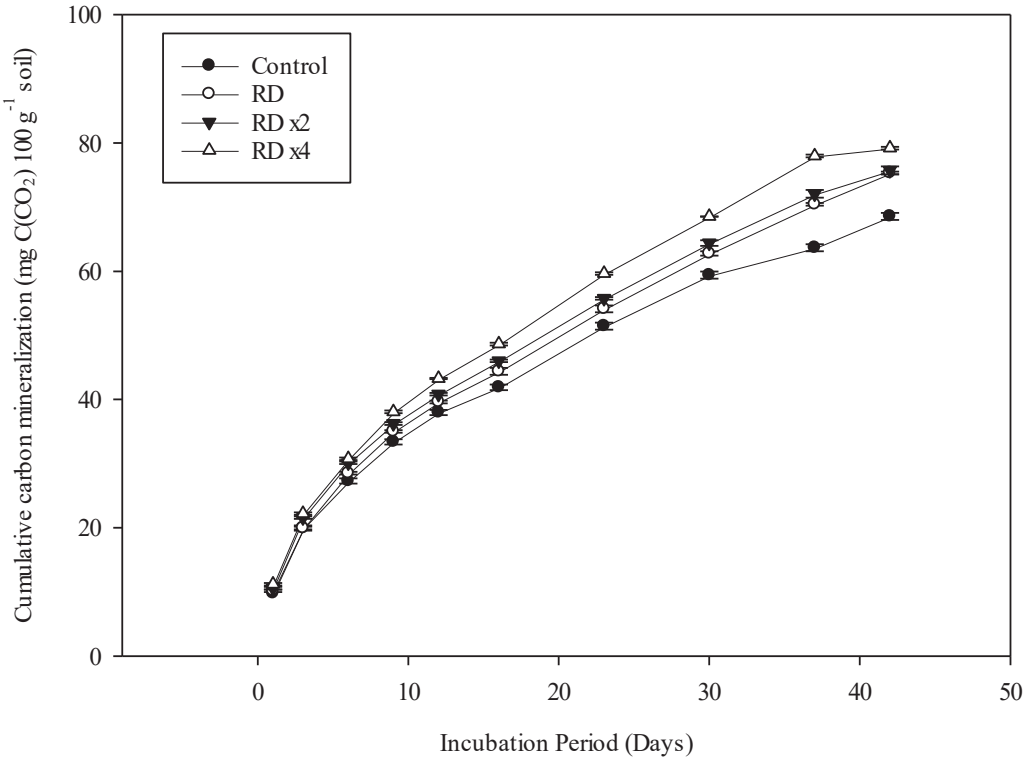


Figure 3. Cumulative carbon mineralizations of control and doses of fungicide cyprodinil at 28°C (mean± standard error, n=3)

Carbon mineralization rates until 30.day, 37.day and 42.day were given in Figure 4. These rates were in between 3.3% (Control) and 3.8% on 30.day, between 3.5 (Control) and 4.3% on 37.day and between 3.8% (Control) and 4.4% (RD x4) (Fig. 4). In all these days, all cyprodinil doses significantly increased the rate of soil carbon mineralization compared to control ($P<0.05$, Fig. 4).

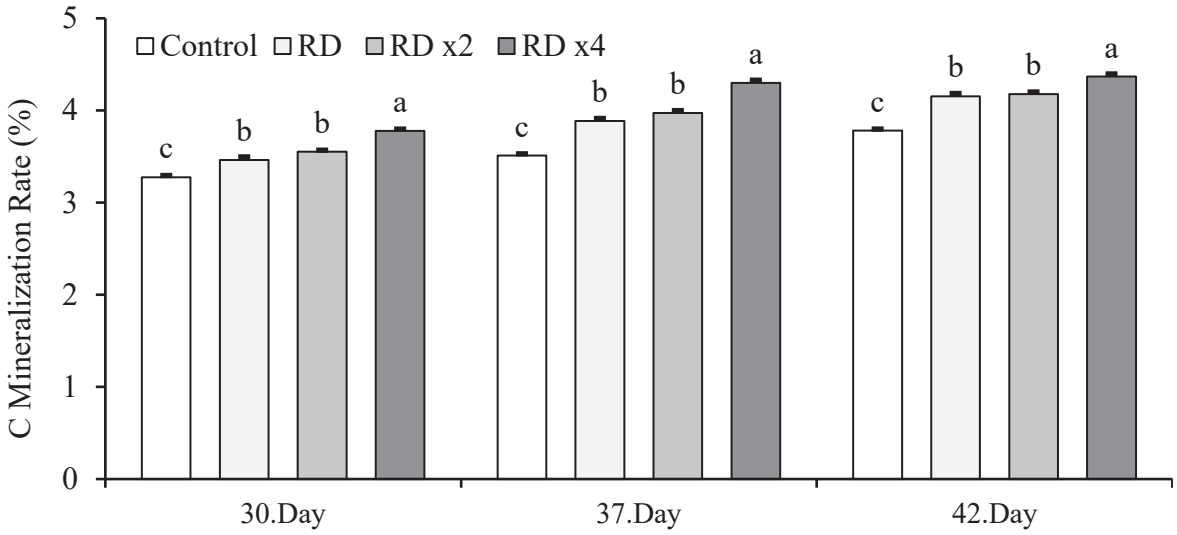


Figure 4. Rate of soil carbon mineralizations (%, mean ± standard error, n=3) of control and doses of fungicide cyprodinil [a, b and c represent significant differences between dosages ($P<0.05$)]

Application of fungicides for certain purpose on soil can kill or inhibit the target and non-target microorganisms. This event could lead to an immediate inhibition of important reactions such as enzyrne reactions, microbial respiration and mineralization. However, the dead microorganisms or fungicides, might serve as a substrate for the other living microorganisms in the beginning. So, they can be released from competition with fungi or antagonistic inhibition via substances produced by fungi. These effects could lead to a rapid flush of microorganism activity would be likely to increase the rates of mineralization of carbon from organic materials in the soil (Uyanoz et al. 2005). Uyanoz et al. (2005) mixed three fungicides with a silty soil at four concentrations [captan, 0, 100, 200, 400 mg active ingredient (a.i.) kg^{-1}; quintozene 0, 36, 72, 144 mg a.i. kg^{-1} and propamocarb hydrochloride 0, 144, 288, 576 mg a.i. L^{-1}] and found that these fungicides decreased soil respiration primarily during the first eight days, but then it started rising toward the end of the incubation.

CONCLUSIONS

It's concluded that recommended field dose of fungicide cyprodinil (300 EC) significantly increased carbon mineralization in a loamy soil. In addition, there were generally no significant differences between RD and RD x2 doses. Significant increase of rate of carbon mineralization in the loamy soil was highest at the dosage of RD x4 in whole experiment. These results may give a suggestion of reasonable and safe use of the pesticide cyprodinil. However, for further confirmation, effects of high recommended doses of cyprodinil on soil microorganisms should be investigated more comprehensively to eliminate its possible side effects in soil. Therefore, it's suggested that the further researches require the trials with soils having different organic matter, texture and pH and with more fungicides type, dose and incubation period under laboratory, greenhouse and field conditions.

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