EFFECTS OF FUNGICIDE TEBUCONAZOLE ON SOIL MICROBIAL RESPIRATION

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ABSTRACT

Application of pesticides has been one of the unavoidable ways to eliminate different types of disease, insect pest, and weeds in agricultural production. On the other hand, environmental problems including water and soil pollution may occur due to the usage of high frequency of pesticides. Tebuconazole is a fungicide (250 g active ingredient/l) that has been used on wheat, tomato, pepper, eggplant, cucumber, apricot and citrus in Turkey. A short term experiment was carried out to ascertain the effects of different recommended doses (RD) of fungicide tebuconazole [0 (Control), 8.9 (RD), 17.8 (RD x2) and 35.6 mg/ kg soil (RD x4)] on microbial respiration 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 tebuconazole usage. After determination of some soil physical and chemical properties, soil was 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. In general, there were no significant differences between control, RD and RD x2 in all measured days. In contrast, RD x4 significantly increased soil microbial respiration until 23th day ($P<0.05$). Cumulative microbial respiration (mg CO_2/100 g soil) were between 250.87 (Control) and 294.17 (RD x4). No significant differences were found between control and RD while RD x2 and RD x4 significantly increased soil microbial respiration at the end of incubation period ($P<0.05$). Percentages of increased respiration compared to the control were 8.93% for RD x2 and 17.26% RD x4. It's suggested that recommended dose of fungicide tebuconazole had no negative effect on microorganisms in soil that was sampled for this study. In conclusion, RD x2 and RD x4 of this fungicide may be an energy source for soil microorganisms for maintaining their growth and metabolisms.

Keywords: fungicide, soil carbon mineralization, tebuconazole, soil microbial activity

1. INTRODUCTION

Microbial metabolic activity in soil is usually measured based on the catabolic respiratory activity since respiration is an important process in the global carbon cycle and of critical importance in the partitioning of energy in soil. Consumption of O_2 and liberation of CO_2 are maintained by aerobic respiratory processes and these events lead to a loss of C from ecosystems to the atmosphere. Organic matter and plant exudates are transformed and respiratory processes of microorganisms mineralize nutrients in the soil. The CO_2 evolution in

soil has been the focus of research because storage, sequestration or liberation of soil carbon is considered to be related to climate change in recent years (Dilly, 2005).

Intensive usage of pesticides in world was accepted as a common application in modern agriculture (Yang et al., 2007). Many studies showed that less than 0.3% of pesticides can reach target organism (Pimentel, 1995) and 99.7% of these pesticides can be released in the environment that these chemicals can become a potential hazard for non-target organisms including humans (Munoz-Leoz et al., 2011). An important part of pesticide can usually reach to the soil and can be underwent to biological and physicochemical transformations (Bending et al., 2006). Therefore, microbial degradation is one of the main pathways in removal of pesticides in soils and this process maintains persistence and susceptibility to leaching of pesticides (Bending et al., 2006). On the other hand, pesticides can apply non-target effects on soil microbial communities and thus, can influence soil health negatively (Lupwayi et al., 2010; Monkiedje & Spiteller, 2002). It was reported that soil microbial respiration one of the most important indicators for detection of factors affecting soil health negatively (Hernandez-Allica et al., 2006; Mijangos et al., 2009).

Many of these agricultural chemicals can negatively affect the populations and functions of microorganisms in soils. Decrease in bacterial and fungal biomass can lead to decrease the activity of soil respiration, affect the soil enzymes and microbial diversity and decline the carbon and nitrogen turnover rates (Johnsen et al., 2001; Lin & Brookes, 1999; Monkiedje & Spiteller, 2002). However, it is difficult to report a general conclusion for the effects of pesticides on soil microorganisms due to the affection of factors like soil structure and texture, pH, organic matter content, temperature and moisture on pesticide behavior and fate in soils (Beulke & Malkomes, 2001; Kim et al., 2002; Monkiedje & Spiteller, 2002). Persistence, availability and toxicological activities of pesticides as well as microbial metabolism can strongly depend on these factors (Cycon et al., 2006)

Tebuconazole ($C_{16}H_{22}ClN_3O$, molecular weight: 307.82 g/mol) is a broad spectrum triazole fungicide that has been applied in fruits, cereals and vegetables for disease control (Munoz-Leoz et al., 2011). Gorur & Akgul (2019) reported that dipping of grapevine cuttings into the fungicide suspensions like tebuconazole is one of the control parameters to produce healthy grapevine plants in nurseries. This fungicide has a relationship in high organic carbon-water bonding and it was reported that its half-life was between 49 and 610 days in aerobic conditions (Strickland et al., 2004).

To eliminate the potential side effects of on the soil ecosystem, this important research is required to gain a better understanding of effects of different doses of fungicide tebuconazole on soil microbial activity and to provide a more accurate evaluation of its effect on soil microbial respiration. The primary goal of this study was to quantify the soil CO_2 respiration under laboratory conditions when tebuconazole was added at three different concentrations of recommended field dose [0 (Control),8.9 (RD), 17.8 (RD x2) and 35.6 (RD x4) mg/ kg soil] to an agricultural soil with no previous history of fungicide application. It was hypothesized that application of tebuconazole more than recommended field dose would decrease soil microbial respiration.

2. MATERIAL AND METHODS

2.1. Soil Characterization

Soil samples that were not previously exposed to fungicide tebuconazole 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).

2.2. Incubation Experiment

Soil samples (100 g) were placed in 750 ml incubation vessels and mixed with fungicide tebuconazole at the recommended field dose and its 2 and 4 folds [8.9 (RD), 17.8 (RD x2) and 35.6 mg/ kg soil (RD x4)]. The amount used in RD treatment was the recommended field rate for tebuconazole (250 g active ingredient/l) that was written its etiquette with the assumption that the soil weight was 1.4×10^6 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 day. 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 beakers, 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 and 42 (Alef, 1995). Cumulative soil microbial respiration (mg CO_2/100 g soil) was calculated by summing up all CO_2 measured days until end of incubation period.

2.3. Statistical Analysis

Tukey honestly significant difference (Tukey HSD) in analysis of variance (ANOVA) was performed to determine the differences in soil microbial respirations between treatments (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.

3. 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. Soil carbon/nitrogen ratio was 11.20.

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 (L)
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

3.1. Soil CO_2 Evolution

This study evaluated the ecotoxicological impact of a commonly used fungicide called tebuconazole on soil microbial respiration at laboratory scale. Soil CO_2 respirations at 28°C were given as between 1.day and 12.day in Figure 1 and as between 16.day and 42.day in Figure 2. These respirations (mg CO_2/100 g soil) were between 14.37 and 37.33 for control, 14.19 and 39.57 for RD, between 16.05 and 38.08 for RD x2 and between 8.96 and 44.80 for RD x4 (Fig. 1 and 2).

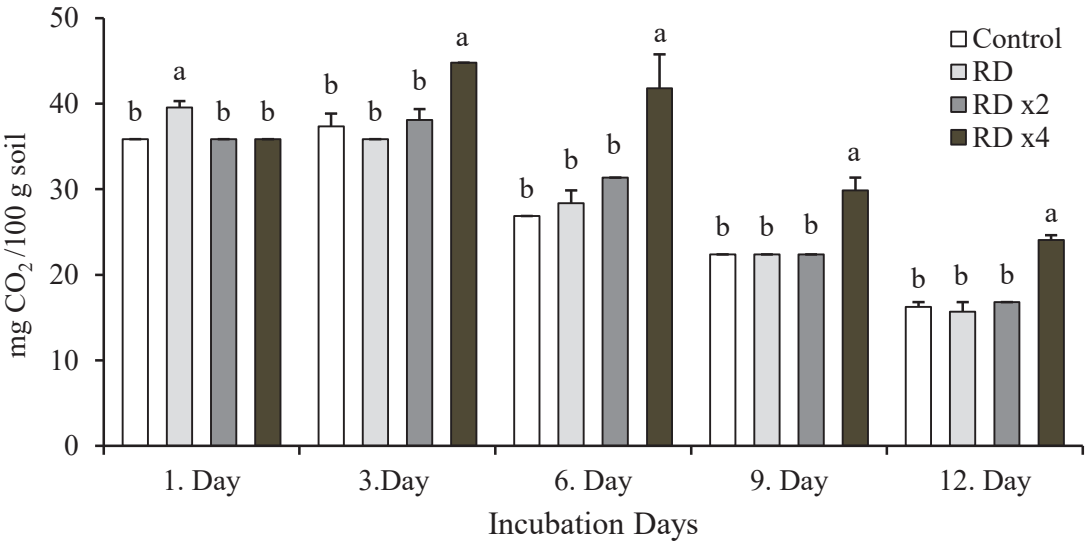


Figure 1. Soil microbial respirations under the effects of fungicide tebuconazole between 1. day and 12. day at 28°C [mean± standard error, n=3, letters (a and b) indicate significant differences between means (P<0.05)]

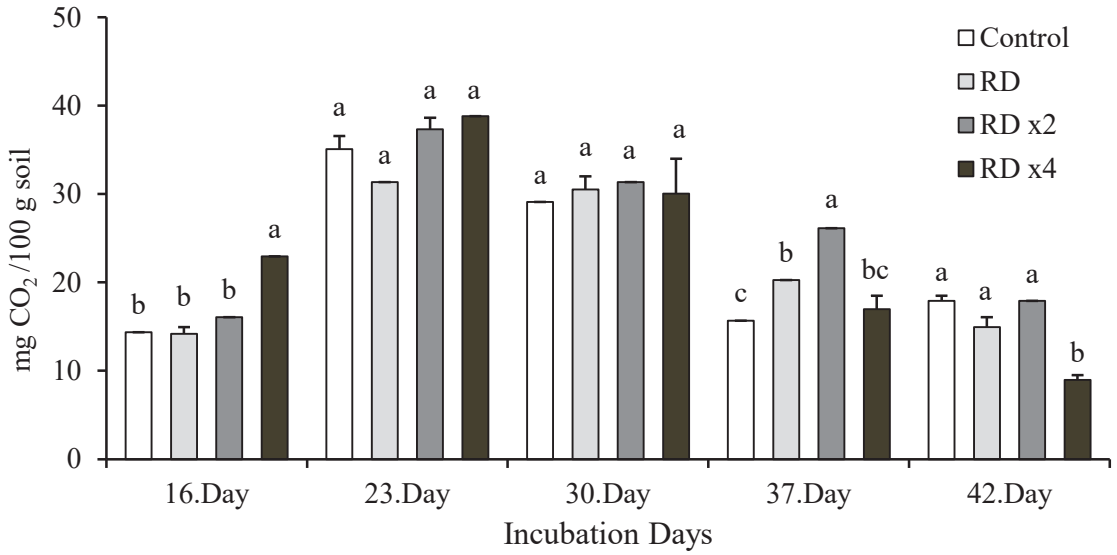


Figure 2. Soil microbial respirations under the effects of fungicide tebuconazole between 16. Day and 42. Day 28°C [mean± standard error, n=3, letters (a, b and c) indicate significant differences between means (P<0.05)]

There were found no significant differences until 16.day between control, RD and RD x2 treatments except on 1.day (Fig. 1). RD significantly increased soil CO_2 respiration more than control, RD x2 and RD x4 on 1.day (P<0.05, Fig. 1). After first day, RD x4 significantly increased this respiration for more than 20% compared to control until 16. day (Fig. 1). Between 16. day and 42.day only significant differences were found among control, RD and RD x2 on 37. day (P<0.05, Fig. 2). In contrast, microbial respiration under RD x4 was variable as following after 16. day: 1) significantly higher than all treatments on 16.Day (P<0.05) 2) showed similarity with control on 23., 30., and 37. Days 3) significantly lowered soil respiration compared to control on 42. day (P<0.05).

The community of soil organisms includes a large number of species of bacteria, archaea, fungi, nematodes, arthropods, and earthworms but bacterial and fungal communities generally dominate biogeochemical activity. Microorganisms respire CO_2 back to the atmosphere during the process of decomposition and some of the carbon that flows through the microbial biomass becomes stabilized into soil organic matter, the critical long-term carbon sink (Clemmensen et al., 2013; Lehmann & Kleber, 2015). Many synthetic compounds are sufficiently similar to naturally occurring compounds. They can be metabolized by microorganisms in the environment by giving the right conditions and adequate time,. The term “biodegradation” is used to describe a biological process that converts an organic compound to CO_2, H_2O, and/or inorganic salt(s) or, sometimes, to an intermediate or a dead-end product(s) that could be higher or lower in toxicity than the parent compound. To minimize ambiguity in terminology, the term “mineralization” describes the complete breakdown of organic compounds to CO_2, H_2O, and/or inorganic salt(s) that are present in the original compounds (Van Elsas et al., 2019).

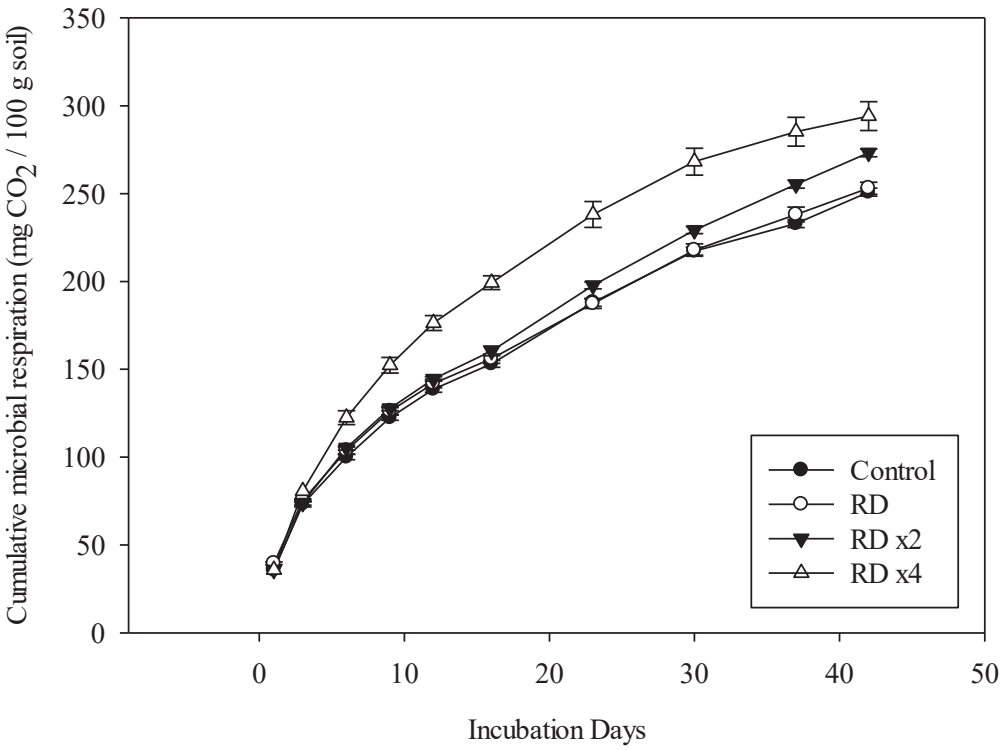


Figure 3. Cumulative soil microbial respirations of fungicide treatments and control for 42 days at 28°C (mean± standard error, n=3)

Cumulative microbial respirations (mg CO_2 /100 g soil) were found as following: 250.87 for control, 253.11 for RD, 273.26 for RD x2 and 294.17 for RD x4 (Fig. 3) No significant differences were found between control and RD. In contrast, RD x2 and RD x4 significantly stimulated soil microbial activity at the end of incubation period ($P<0.05$).

Cycon et al. (2006) reported that three different doses of tebuconazole (2.7, 15.5 and 270 mg/kg soil) generally stimulated substrate induced respiration in a sandy loam soil. In contrast, Munoz-Leoz et al. (2011) reported that three different doses of tebuconazole (5, 50 and 500 mg/kg dry weight of soil) decreased soil microbial biomass and activities in clay sandy soil. It was reported that tebuconazole had showed a moderate to high persistence in the laboratory study and half-lives (DT_{50}) of tebuconazole in soils were 60.7 days for RD treatment and 74.8 days for RD x2 treatment (Papadopoulou et al., 2016). On the other hand, evaluations of European Food Safety Authority (EFSA) declared and reported that the fungicide does not cause an unacceptable risk for soil microorganisms based on carbon and nitrogen mineralization tests (EFSA, 2014). Our results in this study showed with EFSA evaluation report that recommended field dose (RD) and 2 and 4 folds of RD had no negative effect on microbial respiration in a loamy soil. In contrast to findings in this study and EFSA report on tebuconazole, Storck et al. (2018) reported that slight but significant and transient effect of tebuconazole on the composition of the bacterial community in the field experiment.

4. CONCLUSIONS

In this study, recommended field dose of fungicide tebuconazole had no negative effect on microbial respiration in a loamy soil. In addition, higher doses of this fungicide (RD x2 and RD x4) generally enhanced soil microbial activity.

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