Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Biology

The Effects on Soil Carbon Mineralization of Different Doses of Epoxiconazole and Carbendazim Suspension used in Wheat Cultivation

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Original Research Article

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Article History
Received: 03.03.2018
Accepted: 18.03.2018
Published:30.03.2018

DOI:
10.36347/sajb.2018.v06i03.005

Abstract: In this study, the effects on soil carbon mineralization of different doses [recommended dose (RD), × 2RD and × 4RD] of Epoxiconazole + Carbendazim fungicide suspension used for destroying the powdery mildew and yellow rust diseases in the cultivation of wheat being a common agriculture product in Osmaniye (Turkey) were determined in soils before and after wheat spraying and the campus soil of Osmaniye Korkut Ata University. Carbon mineralization of three different soils moistered at 80% of the field capacity were observed under controlled conditions (28 °C and 45 days) and CO_2 respiration method. At the end of the 45 days incubation period, the highest and statistically significant value of cumulative carbon mineralization was observed in wheat soil sampled after fungicide spraying (AWS × 2RD). This value (7.756 mg $C(CO_2)$/100 g) was significantly different in both within own group and among the other groups ($P < 0.001$). This result showed that Epoxiconazole + Carbendazim fungicide suspension added at × 2RD level was only used as a carbon source for microorganisms in the research soils. In the light of this finding, it might be said that the amount of carbon added into the soil with other doses of this fungicide was not enough to increase microorganism activity.
Keywords: Carbon source, Epoxiconazole plus Carbendazim, Fungucide, Mineralization.

INTRODUCTION

Environmental pollution appears to be an important problem which increasingly continues [1]. Soil pollution is also the most important one among these, and chemical wastes and pesticides take place among the causes of pollution [2, 3].

Industry and technology which have developed together with the increasing world population since the beginning of the 20th century have brought forward intense agricultural practices to obtain more product from a unit area. Accordingly, it has become inevitable to use agricultural pesticides to protect plants and plant products against pests, disease factors and effects of weeds and to obtain a quality and rich harvest. Pesticides are synthetic organic compounds used to destroy undesired harmful organisms in products [4]. All kinds of medicine used for plant protection, preparations and substances used in their production are included in this group. In addition to the advantages of pesticides, it has been determined that they cause harm to the ecosystem and human health as a result of long-term use [5-7].

Due to the fact that wheat [*Triticum aestivum* L. (Poaceae)] is a valuable and cheap food product in addition to its containing carbohydrate, starch, protein, some vitamins and mineral substances in the composition, it is an indispensable nutritional source for the rapidly increasing world population. Epoxiconazole ($C_{17}H_{13}C_1FN_3O$) + Carbendazim ($C_9H_9N_3O_2$) suspension is a commonly used fungicide to eliminate ashing and yellow rust diseases in wheat growing. This suspension is commonly used in wheat cultivation in Turkey since it provides effective results and an increase in wheat yield [8]. It is observed that some farmers apply two times more than the recommended dose of pesticides to their fields and sometimes they mix two or three different pesticides to obtain a better product [9]. It has been revealed in the studies conducted that such practices negatively affect microorganism activities (respiration, mineralization, enzyme activity, etc.) in the soil [10-12].

Soil respiration is a complex way affected by the temperature, moisture, soil properties, quality and quantity of decomposing organic substrates [13-15]. Soil microorganisms carry out a wide range processes that are important for soil fertility and health in both natural and managed agricultural soils [16]. As soil microorganisms play a key role in cycling of nutrient, crop residues form the essential supply of carbon (energy source) and nutrients for microbial activities.

Activities of soil microorganism are also influenced by environmental factors such as temperature, moisture, pH, amount and presence of agricultural chemicals (e.g. herbicides, insecticides) in the soils [17, 18]. Long-term and overdose applications of agricultural chemicals have serious impacts on soil biology that may lead to change total microbial population and their biochemical activities [9, 19].

In this study, it was aimed to determine the effects on the soil carbon mineralization (28 °C, 45 days) of different doses [Recommended Dose (RD), × 2RD and × 4RD] of 125 g/l Epoxiconazole + 125 g/l Carbendazim fungicide suspension applied to the wheat [*Triticum aestivum* L. (Poaceae)] widely cultivated in Osmaniye (Turkey) to eliminate some pathogenic fungi.

MATERIALS AND METHODS

This study was carried out in Çardak village of Osmaniye city (Turkey), which is characterized by a semiarid Mediterranean climate, having a mean annual precipitation of 808 mm and mean annual temperature of 18.2 °C for the last 27 years. Soil samples were taken from a wheat field of Çardak village two times before and after 125 g/l Epoxiconazole + 125 g/l Carbendazim fungicide suspension application to the field soils. Third soil was sampled from Osmaniye Korkut Ata University (OKU) campus area because of being an area not applied any pesticides or chemical plant nutrients. Five superficial soil samples from the 10 cm depths of both wheat field (before and after pesticide application) and OKU campus soils were collected from each of four corners and the medium of these areas according to its shape in between March (wheat soil before pesticide application) and May (wheat soil after pesticide application and campus soil) of 2016 years. Five soils of each of three separate samples were respectively mixed, homogenized, and regarded as a representative sample. After removing recognizable plant debris, samples were air-dried and sieved through a 2-mm mesh sieve.

The soil texture was determined with a Bouyoucos hydrometer [20], field capacity (%) with a vacuum pump at 1/3 atmospheric pressure [21] and $CaCO_3$ content (%) with a Scheibler calcimeter [22]. Soil pH and electrical conductivity (EC) were determined in a 1:1 soil to distilled water ratio [23, 24] using microprocessor pH-meter (Van London Phoenix Electrodes, USA). Soil organic carbon content (%) was measured by the Anne method [25] and organic nitrogen content (%) by the Kjeldahl method [25].

125 g/l Epoxiconazole ($C_{17}H_{13}C_1FN_3O$)+ 125 g/l Carbendazim ($C_9H_9N_3O_2$) suspension is a fungicide which was sold as licensed in Turkey and used to eliminate epidemic fungus diseases such as yellow rust (*Puccinia striiformis* West.) and ashing [*Erysiphe graminis* (DC.) Wint.] in the wheat farming. The recommended dose (RD) and 2 and 4 folds of this dose of 125 g/l Epoxiconazole + 125 g/l Carbendazim suspension (Trading name: ACS Swot SCS; Production firm: Asia Crop Science) was calculated according to volume weight (1.40 g/cm) and added to the soils at the amount (100 g/d) stated on the fungicide box for the carbon mineralization treatments. This fungicide suspension were not added to the control soil because of observing the clearer effects of 125 g/l Epoxiconazole + 125 g/l Carbendazim suspension on soil carbon mineralization.

Soil samples were placed into incubation vessels (750 ml) for carbon mineralization and the fungicide dissolved in distilled water was added. The final moisture contents of soils were adjusted to 80% of their own field capacity before incubation at 28 °C over 45 days [26]. CO_2 derived from microbial activities was absorbed in 10 ml of 1 M NaOH 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 once every 3 days [27] by titration with 0.05 M HCl. Empty vessels were used as blanks. The rate (%) of carbon mineralization was calculated by dividing the cumulative amount of $C(CO_2)$ produced in 45 days into total organic carbon content of the soils.

The LSD analysis (Least Significant Difference) and Duncan's test were used in the multiple comparisons to reveal the presence of a statistically significant difference among three different fungicide doses (×RD, ×2RD and ×4RD) added to the soils (BWS: wheat soil sampled before fungicide application, AWS: wheat soil sampled after fungicide application and CS: campus soil of Osmaniye Korkut Ata University). Three replicates were used for each combined soil for statistical comparisons and their mean values ± standart error (SE) in tables and figures. Data were analyzed by a series of analyses of variance [28]. Differences between data were assumed significant at $P < 0.05$. All statistical analyses were carried out using SPSS 11.5.

RESULTS AND DISCUSSION

Physico-chemical properties of soils used in carbon mineralization experiments were presented in Table 1. All of soil samples were slightly alkaline and loam-textured. There were not significant differences among three soil samples in point of saturation (%), texture type, field capacity (%), pH, C content (%) and C/N ratio ($P>0.05$). But $CaCO_3$ content (%) and EC (%) were statistically different from the each other among the all soils ($P \leq 0.05$). N content (%) of campus soil is the significantly highest in both soils before and after fungicide spraying (Table 1).

Table-1: Some physico-chemical properties (mean ± standard error, n=3) of wheat soil sampled before and after fungicide spraying and campus soil of Korkut Ata University in Osmaniye (FC: Field Capacity)

Analysis	Wheat soil before fungicide spraying				Wheat soil after fungicide spraying				Campus soil			
Saturation (%)	45.0	±	0.01	a	43.1	±	0.03	a	49.2	±	0.05	a
Texture type	Loam (L)				Loam (L)				Loam (L)			
FC (%)	20.6	±	1.22	a	17.5	±	0.38	a	20.18	±	0.90	a
$CaCO_3$ (%)	1.77	±	0.02	a	2.13	±	0.03	b	4.37	±	0.03	c
pH	7.70	±	0.00	a	7.70	±	0.00	a	7.70	±	0.00	a
EC (%)	0.59	±	0.00	a	0.48	±	0.00	b	0.53	±	0.00	c
C (%)	1.71	±	0.43	a	1.13	±	0.14	a	1.56	±	0.29	a
N (%)	0.16	±	0.03	b	0.13	±	0.01	a	0.31	±	0.06	c
C/N ratio	10.4	±	1.69	a	8.58	±	0.71	a	5.76	±	2.24	a

There was no significant difference among four various doses (control, × RD, × 2RD and × 4RD) of wheat soil (BWS) sampled before fungicide spraying in terms of carbon mineralization ($P > 0.05$, Figure 1). The carbon mineralization of control of wheat soil (AWS) sampled after fungicide spraying was significantly lower than AWS × 2RD ($P = 0.025$, Figure 2). But the other treatments had similar results and were not found significant differences among each other of wheat soil added fungicide. This result suggested that 2 fold additions of Epoxiconazole and Carbendazim fungicide suspension recommended for the wheat field was used as a carbon source for microorganisms living in the soil. When this dose was compared with the other doses (control, AWS × RD and AWS × 4RD), AWS × 2RD might be a threshold value to be used as a carbon source for soil microorganisms.

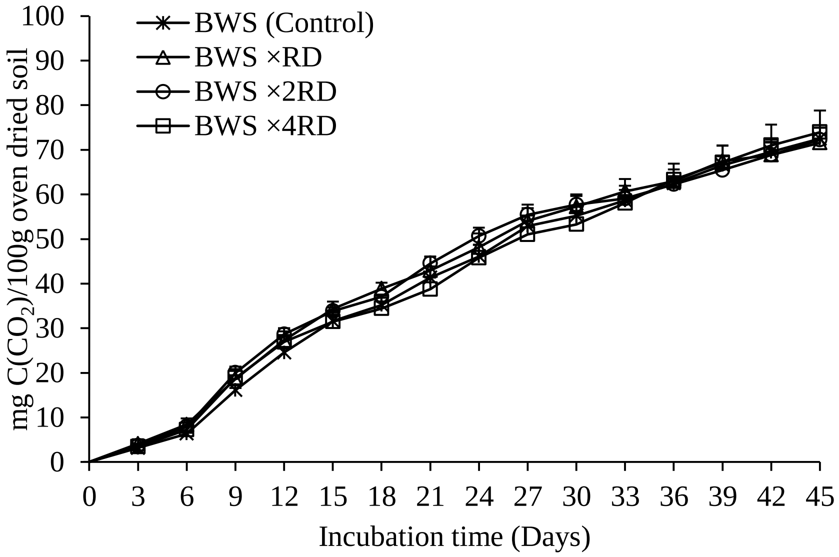

Fig-1: Carbon mineralization [mg $C(CO_2)$/100 g] of control and ×RD, ×2RD and ×4RD additions of wheat soil sampled before fungicide spraying (BWS) in controlled conditions [(28 °C, 80% of own field capacity, (mean ± SE, *n* =3)]

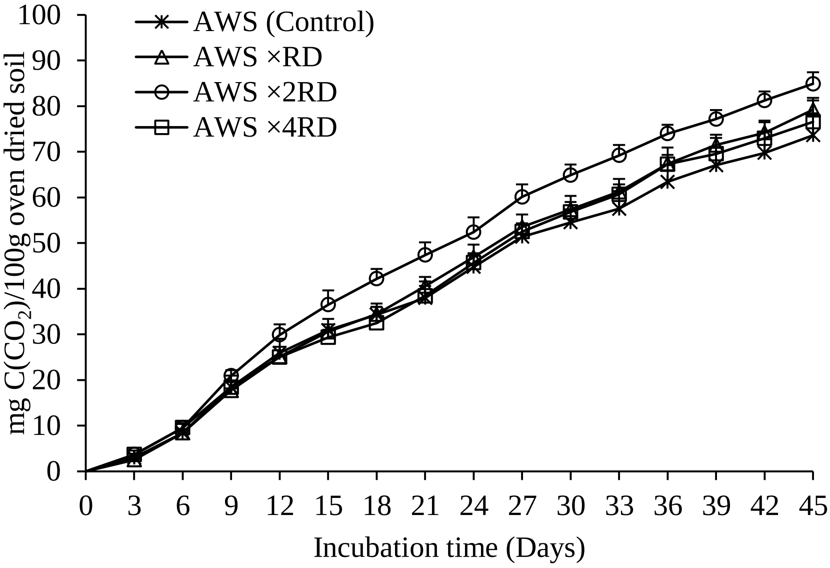

Fig-2: Carbon mineralization [mg C(CO_2)/100 g] of control and ×RD, ×2RD and ×4RD additions of wheat soil sampled after fungicide spraying (AWS) in controlled conditions [(28 °C, 80% of own field capacity, (mean ± SE, *n* =3)]

The carbon mineralization of control group without fungicide of the campus soil (CS) was found significantly lower than CS × RD and CS × 2RD ($P = 0.024$ and $P = 0.013$, respectively, Figure 3). CS × 4RD was also significantly lower than the CS × 2RD after especially beginning of the 36th day of the incubation periods ($P = 0.040$). Similarly, some researchers found that cumulative soil carbon mineralization increased with increasing glyphosate rate in their study [19]. Our results showed that it was found to be considerably significant in terms of revealing the effect of fungicide suspension added to a soil cleared from pesticides on microorganisms more clearly. In fact, the fungicide added to clean soil did not have a negative effect according to these results. It has been indicated in different studies that pesticides are used by microorganisms as a carbon or nutritional source [9, 29, 30]. The highest cumulative carbon mineralization for a 45-day period of incubation was observed in the wheat soil treated with twice of the recommended dose (AWS × 2RD, 7.756 mg C(CO_2)/100 g). This result was found to be different at a statistically significant level both within the group (AWS) and compared to the other two groups (BWS and CS) ($P < 0.001$).

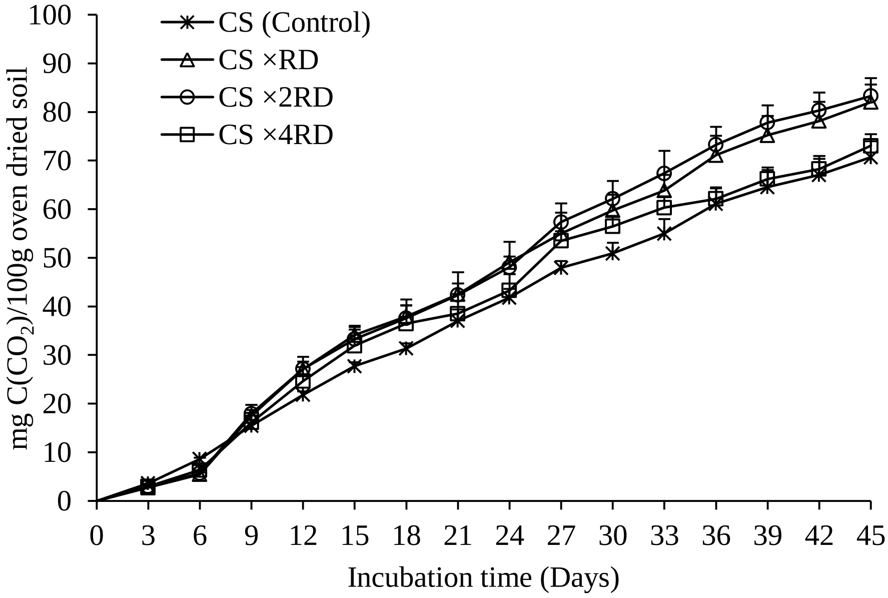

Fig-3: Carbon mineralization [mg C(CO_2)/100 g] of control and ×RD, ×2RD and ×4RD additions of campus soil (CS) of Osmaniye Korkut Ata University in controlled conditions [(28 °C, 80% of own field capacity, (mean ± SE, *n* =3)]

Upon assessing the soil before wheat spraying (BWS) within itself in terms of the carbon mineralization ratios, no statistically significant difference was observed between the different doses and control (P > 0.05, Figure 4). Upon assessing all doses of the BWS among the across groups, it was found to be low at a statistically significant level in almost all of the other two groups (AWS and CS, P = 0.000). Upon assessing the treated wheat soil (AWS) within itself in terms of the carbon mineralization rates, twice the recommended dose was found to be significantly higher than the control and AWS × 4RD (P = 0.31 and P = 0.018, respectively). The difference between the control, AWS × RD and AWS × 4 RD is not statistically significant (P > 0.05). When all doses of AWS were compared with the other two groups (BWS and CS), they were found to be statistically significantly higher when compared to both these groups (P=0.000 in all multiple comparisons, Figure 4). Upon assessing the campus soil (CS) within itself in terms of the carbon mineralization rates, no statistically significant difference was observed between all doses and control (P > 0.05). However, when this condition was compared with the other two groups (BWS and AWS), it was not the same, and the CS × RD and the CS × 2RD were found to be significantly higher compared to the control and all the other doses of BWS (P = 0.000 in all multiple comparisons). The other two doses (control and × 4RD) of CS have similar carbon mineralization rates with all doses and control of BWS, and the difference between them was insignificant (P > 0.05, Figure 4).

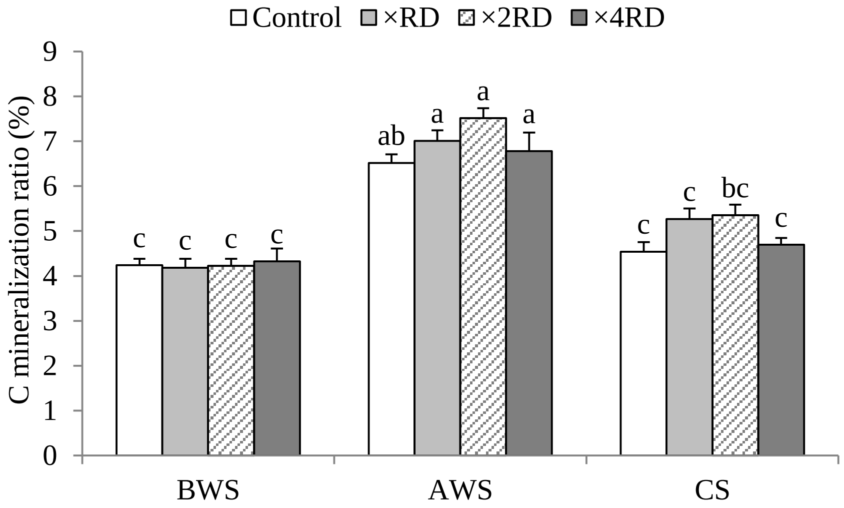

Fig-4: Carbon mineralization ratios (%, mean ± SE, *n* =3) of control, ×RD, ×2RD and ×4RD additions of wheat soil sampled before and after fungicide spraying (BWS and AWS) and campus soil (CS). Different letters (a, b, c) represent significant differences between both different dosages and groups (P < 0.05)

It is known that microorganisms are more sensitive to pesticide changes than the other organisms in their environments. Nitrate bacteria were negatively affected by the presence of imazamox herbicide in soils [30]. Another study result showed that an increase of 10–15% in the CO_2 evolved and a 9–19% increase in FDA hydrolyses in the presence of glyphosate compared with the same type of soil which had never received glyphosate [31]. This study results showed that presence of Epoxiconazole + Carbendazim fungicide suspension in BWS, AWS and CS slightly affected on soil microorganism activity. Two fold level of the recommended dose of this fungicide suspension added to the all research soils was only used as a carbon source by microorganisms. In the light of this finding, it might be said that the amount of carbon added into the soils together with the other doses of this funguicide was not enough to increase microorganism activity in BWS, AWS and CS.

CONCLUSION

Carbon mineralization values of BWS treatments showed that microorganisms did not affect from the first fungicide application and overdose. AWS treatments showed that × 2RD of Epoxiconazole and Carbendazim suspension of microorganisms used as a carbon source better than the other doses. Because of being clear and not exposed pesticide application before, the carbon mineralization results of CS treatments was clearer than the wheat soils sampled before and after fungicide spraying. Microorganisms in the campus soil met the first time with a foreign matter in their environment and used × RD and × 2RD as a carbon source. But it was observed that × 4RD level of this fungicide had stifling effect [19] for the microorganisms in the campus soil.

ACKNOWLEDGEMENTS

We thank Professor Cengiz DARICI for calculation of fungicide amounts added to the soils in the project. This project numbered OKUBAP-2016-

PT3-010 was funded by Research and Application Center in Osmaniye Korkut Ata University.

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