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Abstract: Glyphosate isopropylamine and trifluralin are herbicides widely used in Turkish agriculture. The recommended field dose (RFD) (480 g of active ingredient l^{-1} for both glyphosate and trifluralin) and 2 × RFD of these herbicides were added to the soil of olive trees (*Olea europaea* L., Oleaceae) growing on the Çukurova University Campus (Adana) under Mediterranean climate conditions in order to determine their effects on soil microbial activity as measured by carbon mineralization. Carbon mineralization of all samples was determined by the CO_2 respiration method over 30 days (28 °C, constant moisture). When the RFD and 2 × RFD of glyphosate were compared to a control treatment with no herbicide, they significantly stimulated carbon mineralization at the end of the 30 days ($P < 0.001$), but the RFD and 2 × RFD of trifluralin were not statistically different from the control ($P > 0.05$). Based on these results, it is possible to conclude that soil microorganisms are capable of using glyphosate as a carbon source.

Key Words: Carbon mineralization, glyphosate isopropylamine, herbicide, trifluralin

Zeytin Topraklarının Karbon Mineralizasyonuna Glyphosate Isopropylamine ve Trifluralin'in Etkileri

Özet: Glyphosate isopropylamine ve trifluralin Türkiye'nin tarımsal alanlarında yaygın olarak kullanılan herbisitlerdir. Bu herbisitlerin önerilen tarla dozu (hem glyphosate hem de trifluralin için 480 g l^{-1}) ve bu dozun 2 katı Akdeniz ikliminin etkisi altındaki Çukurova Üniversitesi kampus (Adana) alanında yetiştirilen zeytin ağacı (*Olea europaea* L., Oleaceae) toprağına karbon mineralizasyon ölçümüyle toprak mikrobiyal aktivitesine etkilerini belirlemek amacıyla ilave edilmiştir. Tüm örneklerin C mineralizasyonu 30 gün boyunca (28 °C, sabit nem) CO_2 respirasyon yöntemiyle belirlenmiştir. Glyphosate'in önerilen tarla dozu ve bu dozun 2 katı, herbisit içermeyen kontrolle kıyaslandığında C mineralizasyonu 30 günün sonunda anlamlı düzeyde artmıştır ($P < 0.001$). Fakat, trifluralinin önerilen tarla dozu ve bu dozun 2 katı kontrolden istatistiksel olarak farklı değildir ($P > 0.05$). Bu sonuçlara dayanarak toprak mikroorganizmalarının glyphosate'i karbon kaynağı olarak kullanma yeteneğine sahip olduğu sonucuna varmak mümkündür.

Anahtar Sözcükler: Karbon mineralizasyonu, glyphosate isopropylamine, herbisit, trifluralin

Introduction

Glyphosate isopropylamine [*N*-(phosphonomethyl)-glycine] and trifluralin (a,a,a-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) are herbicides widely used in agriculture (Zirai Mücadele Teknik Talimatları, 1995; Vencill, 2002). Trifluralin keeps crops weed free in the early crop stages by controlling annual grass weeds and certain small-seeded broad-leaved weeds (Malterre et al., 1997; Vencill, 2002; Strynar et al., 2004; Tiryaki et al., 2004). The value of glyphosate has been compromised in some fields where weed populations have developed resistance or the presence of tolerant species has increased (Mueller et al., 2005).

From an environmental perspective, the use of glyphosate would be preferred over other herbicides that exhibit greater soil mobilities, half-lives, or mammalian toxicities. However, glyphosate-based weed control systems have resulted in extensive use of the herbicide, with multiple applications in a single growing season becoming common (Mueller et al., 2005). Camper et al. (1980) and Johnston and Camper (1991) studied trifluralin degradation in various types of agricultural soil under aerobic and anaerobic conditions, and found that breakdown was highly dependent on the soil type. In a soil used for the disposal of various pesticides (diuron, trifluralin, carbofuran), only trifluralin was still detected after many years (Johnston and Camper, 1991).

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Herbicides have been used in some agricultural fields in Turkey (Bukun, 2004; Tiryaki et al., 2004; Uludağ et al., 2006). The herbicide trifluralin has been used on almost all maize and cotton fields in Turkey since the 1970s (Uludağ et al., 2006). Some farmers use the herbicides at 2 × RFD and even use mixtures of 2 or 3 different herbicides to obtain better crops in Turkey. These applications might cause unwanted side effects on non-target organisms in the soil (Busse et al., 2001). The presence of glyphosate or trifluralin in soil may cause changes to the microbial population and activity of a soil (Hang et al., 2001; Busse et al., 2001; Haney et al., 2002a, 2000b; Araújo et al., 2003).

The aim of the present investigation was to comparatively evaluate the effects of RFD and 2 × RFD of glyphosate isopropylamine and trifluralin on the microbial activity of olive tree (*Olea europaea* L., Oleaceae) soils (without a previous application of glyphosate isopropylamine and trifluralin) by measuring carbon mineralization in the Eastern Mediterranean region of Turkey. Because it is well adapted to the Mediterranean climate zone, the olive tree is a good bio-indicator that characterizes this zone. In this study, soils of olive trees were selected for growing in herbicide untreated areas. The trees had been planted 25 years previously in these herbicide untreated areas and grew naturally without human interference.

Materials and Methods

Soil was collected from an unfertilized olive tree plot (0.8 ha) at Çukurova University Campus, Adana (2200 ha; mean annual precipitation: 663 mm; mean annual temperature: 18.7 °C) characterized by semi-arid Mediterranean climate conditions. The precipitation and temperature data of Adana are the average of 50 years (Meteoroloji Bülteni, 2001).

In all, 3 superficial soil samples from the upper 10 cm of olive tree soil were taken from each of 3 corners of the plot in March 2003. The 3 samples were mixed, homogenized, and then considered as a composite and representative sample. After removing recognizable plant debris, these composite samples were air-dried and sieved through a 2-mm mesh sieve. The soil used was loam textured (clay: 10.9%; silt: 50.9%; sand: 38.2%; classified as Alfisols) with a field capacity of 37.1%, soil pH of 7.47 (1:2.5 soil/water), soil $CaCO_3$ of 0.42%, soil organic C of 3.59%, soil organic N of 0.32%, and C/N ratio of 11.2 (Table 1).

Table 1. Some physical and chemical properties (mean ± SE; n = 3) of the olive tree soils (FC: field capacity).

Characteristic	
Clay [< 0.002 mm (%)]	11.0 ± 0.48
Silt [0.02-0.002 mm (%)]	51.0 ± 0.98
Sand [2-0.02 mm (%)]	38.0 ± 0.73
Texture Type	Loam (L)
FC (%)	37.1 ± 0.67
pH	7.47 ± 0.03
CaCO3 (%)	0.42 ± 0.12
C (%)	3.59 ± 0.23
N (%)	0.32 ± 0.02
C/N ratio	11.2 ± 0.24
Volume weight (g cm^{-3})	1.08 ± 0.02

The isopropylamine salt of glyphosate (Roundup®, Takimsan, İstanbul; 480 g of active ingredient l^{-1}) and the salt of trifluralin (Trifil, Polisan, İstanbul; 480 g of active ingredient l^{-1}) were added to the soil at the RFD and 2 × RFD. Rate calculations of glyphosate and trifluralin were based on volume weight (1.03 g cm^{-1}) and a prospectus of these chemicals (6 l ha^{-1} and 2 l ha^{-1}, respectively; 480 g active ingredient l^{-1} for both herbicides). Control treatment without herbicide was included to measure normal soil microbial activity. These values are presented in Table 2.

Soils were placed in 750-ml incubation vessels for carbon mineralization. Herbicides were added to soil samples in 5 ml of distilled water. The final moisture content of the soils was adjusted to 80% of their field capacity before incubation (Schaefer, 1967). The CO_2 produced from microbial respiration was absorbed periodically in 40-ml saturated $Ba(OH)_2$ solution in small tubes, which were placed on the top of the soil in the incubation vessels. The incubation vessels were closed. Empty vessels were used as blanks. The CO_2 produced as a result of microbial respiration was measured every 3 days by titration with oxalic acid (Benlot, 1977). Incubation was carried out in the dark at 28 °C for 30 days in a temperature-controlled incubator. The rate (%) of carbon mineralization carbon was calculated by

Table 2. Glyphosate and trifluralin additions to the olive soils and total C and N added to soil from herbicides.

Doses	Herbicide additions (mg kg^{-1})	Total C added to soil from herbicide (mg kg^{-1})	Total N added to soil from herbicide (mg kg^{-1})	C:N ratios
Control	-	-	-	-
RFDG[1]	47	42	16	2.63
2 × RFDG	94	84	32	2.63
RFDT[2]	16	42	23	1.83
2 × RFDT	32	84	46	1.83

[1] (G): Glyphosate [2] (T): Trifluralin

dividing the cumulative $C(CO_2)$ produced in the 30-day incubation period by total organic carbon.

Soil texture was determined with a Bouyoucos hydrometer (Bouyoucos, 1951) and field water capacity (%) was determined with a vacuum pump with 1/3 atmospheric pressure (Demiralay, 1993). The pH was measured in a 1:2.5 soil-to-water suspension with a pH meter (Jackson, 1958). Organic carbon content (%) was determined by the Anne method (Duchaufour, 1970) and organic nitrogen content (%) by the Kjeldahl method (Duchaufour, 1970).

To determine the differences in carbon mineralization between the herbicides (glyphosate and trifluralin) during the incubation period and at different doses (RFD and 2 × RFD) a repeated measures (General Linear Model) analysis was performed (Kleinbaum et al., 1998). For comparison, 3 replicates were used for each combined soil. Results are given as the mean ± SE of 3 replicates. Differences in the analysis of variance were considered significant at $P \leq 0.05$, 0.01, and 0.001. All statistical analyses were carried out using SPSS v.11.5 (SPSS, Chicago, 2002).

Results

During incubation of the soil samples there were statistically significant differences in carbon mineralization between the herbicide-free control, and glyphosate at the RFD and 2 × RFD at the end of 30 days ($P < 0.001$). Carbon mineralization with trifluralin at the RFD and 2 × RFD compared to the control at the end of the 30 days was not significantly different ($P > 0.05$, Figure 1).

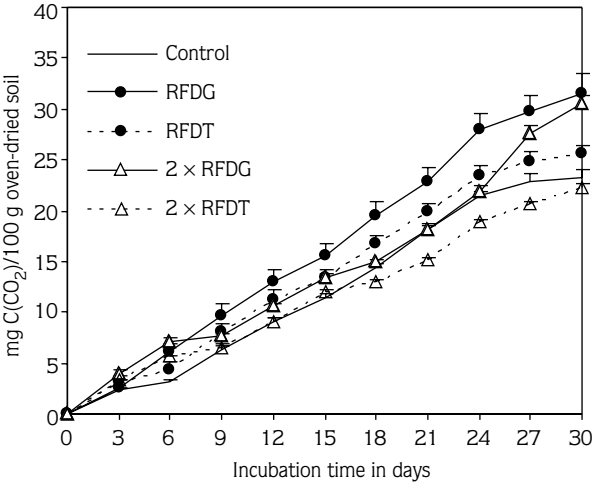

Figure 1. Cumulative carbon mineralized (mean ± SE; n = 3) in the control soil and soils treated with glyphosate at the RFD and 2 × RFD, and trifluralin at the RFD and 2 × RFD on different days.

There were statistically significant differences in carbon mineralization between the soils treated with the 2 herbicides at the RFD and 2 × RFD. These differences were between the RFD of glyphosate and trifluralin ($P < 0.01$), between 2 × RFD of both herbicides ($P < 0.001$), between the RFD of glyphosate and 2 × RFD of trifluralin ($P < 0.001$), and between the RDF of trifluralin and 2 × RFD of glyphosate ($P < 0.05$). Increased microbial activity in the soil with glyphosate was higher than in the soil with trifluralin (Figure 1). Interactions between incubation time and dose, and incubation time and herbicide were significant ($P < 0.001$ for all of them) for microbial respiration. Interaction among incubation time, dose, and herbicide was also significant for carbon mineralized ($P < 0.001$) (Table 3).

Table 3. Results of ANOVA (general linear model) for repeated measures of carbon mineralized (n = 3). Effects of incubation time, dose, and herbicide.

	Type III Sum of Squares	df	Mean Square	F	Sig.
Incubation time	20584.429	1	20584.429	3408.719	< 0.001
Incubation time × dose	7818.114	3	2606.038	431.552	< 0.001
Incubation time × herbicide	126.737	1	126.737	20.987	< 0.001
Incubation time × dose × herbicide	215.378	3	71.793	11.889	< 0.001
Error (time)	434.791	72	6.039		

There was no significant difference between glyphosate at the RFD and 2 × RFD ($P > 0.05$, Figure 1). There was no significant difference between trifluralin at the RFD and 2 × RFD at the end of 30 days ($P > 0.05$, Figure 1).

The rate (%) of carbon mineralization was not statistically different between the control and the RFD and 2 × RFD of both herbicides ($P > 0.05$). The rate of carbon mineralization was not different between the soil treated with glyphosate at the RFD, and soil treated with trifluralin at the RFD and 2 × RFD of glyphosate ($P > 0.05$). However, there was a significant difference between carbon mineralization of the soil treated with glyphosate at the RFD and trifluralin at 2 × RFD ($P < 0.05$) (Figure 2).

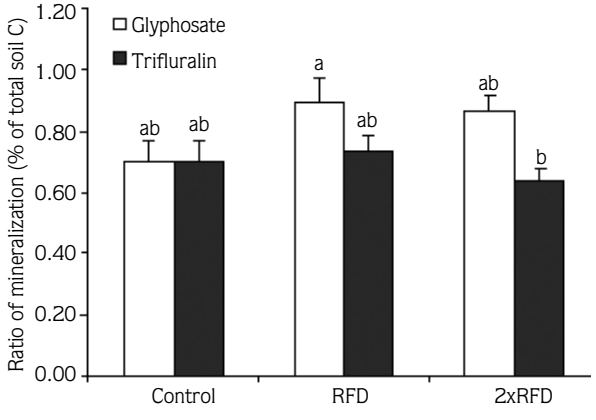

Figure 2. Rate of mineralization (R_m) of organic carbon in the control soil, and soils treated with glyphosate at the RFD, and trifluralin at the RFD and 2 × RFD (mean ± SE; n = 3) during the incubation period (30 days). Different letters denote significant differences between the 2 herbicides ($P \leq 0.05$).

Discussion

It was observed that glyphosate at the RFD and 2 × RFD had a stimulant effect on microbial activity when compared to the control. This result is similar to the results of other studies (Gomez and Sagardoy, 1985; Haney et al., 2002b; Araújo et al., 2003). The stimulation of soil microbial activity in the presence of glyphosate suggests that soil microorganisms are capable of using glyphosate as a carbon source (Busse et al., 2001; Araújo et al., 2003). Wardle and Parkinson (1990) suggest that the decomposition of glyphosate in soil resulted in an increase in microbial activity.

When carbon mineralization in the soil treated with trifluralin at the RFD and 2 × RFD was compared to each other and to the control at the end of the 30 days, no significant differences were observed ($P > 0.05$). This result might be explained by the low solubility of trifluralin in soil. Despite rapid dissipation during the first month after application, trifluralin had a slow degradation rate in the soil, which led to a high persistence of about 10-11 months (Malterre et al., 1997). Messersmith et al. (1971) reported that 3%-5% of ^{14}C-labeled trifluralin (propyl-l-^{14}C) was mineralized to $^{14}CO_2$ during 30 days of incubation in soils after application at a rate of 1 mg g^{-1} of soil. Tiryaki et al. (2004) investigated mineralization of [Ring-UL-^{14}C] trifluralin during 350 days in Gürgelen, Harran, and Ikizce soils. $^{14}CO_2$ increased with time in these 3 soils.

There were significant differences between treatment with both glyphosate and trifluralin at the RFD ($P < 0.01$) and 2 × RFD ($P < 0.001$), between glyphosate at the RFD and trifluralin at 2 × RFD ($P < 0.001$), and between trifluralin at the RDF and glyphosate at 2 × RFD ($P <$

0.05). The addition of glyphosate to the soil significantly increased microbial activity when it was compared to trifluralin. This large increase represents the greater contribution of glyphosate to microbial respiration (Haney et al., 2002b) when compared to trifluralin. It might also be because glyphosate decomposes more easily than trifluralin. Glyphosate is rapidly degraded by soil microbes, presumably by co-metabolism (Sprankle et al., 1975), resulting in increased microbial biomass and activity (Haney et al., 2000; Haney et al., 2002b).

There was no significant difference between treatment with the RFD and 2 × RFD of the herbicides. The RFD and 2 × RFD might be perceived by microorganisms as an available substrate. These results are important for agricultural fields to which glyphosate and trifluralin are applied.

According to the rate (%) of carbon mineralization, there was a significant difference between only glyphosate at the RFD and trifluralin at 2 × RFD. This rate was lowest in soil treated with trifluralin at 2 × RFD (Figure 2). This result might be explained by the slow degradation rate in the soil. Trifluralin might be accumulated in the soil in a 30-day period.

According to these findings, soil carbon mineralization was more sensitive to the addition of glyphosate than trifluralin. Glyphosate may also stimulate mineralization of native organic matter. It can be concluded that soil microbes appeared to prefer glyphosate over trifluralin as a substrate.

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