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Effects of imazamox on soil carbon and nitrogen mineralization under Mediterranean climate

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Abstract: Imazamox is an herbicide intensively used in the peanut fields of Turkey. Carbon and nitrogen mineralizations were determined at 20 °C to evaluate the effects of the herbicide in soils humidified to 60% and 80% of field capacity (FC) over 45 days. Three doses of this herbicide [recommended dose (RD, 10 mg kg^{-1}), 2× RD, and 4× RD] were added to soils without any previous imazamox application (NI) and to peanut soils with previous applications of imazamox (I). Carbon mineralization, determined by CO_2 respiration, at RD of I soil at 80% humidity was higher than that of the other herbicide doses at both 80% and 60% humidities. NO_3-N contents (mg kg^{-1}) of I and NI soil controls were significantly higher than with all herbicide doses at 80% and 60% of FC. Nitrate production decreased with increasing doses of herbicide and was significantly lower in I soil than NI soil at both humidity levels. It may be concluded that the presence of imazamox in both soils was negatively affected by nitrate bacteria.

Key words: Herbicide, humidity, microbial activity, peanut, temperature

1. Introduction

Imazamox ($C_{15}H_{19}N_3O_4$) is an herbicide and amino acid synthesis inhibitor widely used for peanut (*Arachis hypogaea* L., Fabaceae) culture in Osmaniye, Turkey. Due to intensive domestic and industrial consumption of peanut, the dosage of imazamox has critical importance concerning environmental and human health. Imazamox was used in peanut fields at the recommended dose (RD, 10 mg kg^{-1}) in Turkey. The imazamox consumption was determined to be 289 kg for 2002 and 7590 kg for 2008 in Turkey (TMMOB, 2008). Some farmers use herbicides at levels twice or 4 times higher than the RD, and they even mix and apply 2 or more herbicides.

Herbicides, in general, affect microorganisms by causing physiological changes and altering enzymatic production. At higher doses, however, they were found to be responsible for the death of susceptible groups of microorganisms (Cervelli et al., 1978). Soils displayed a general variability of enzyme activities, with invertase being more abundant than urease and phosphatase. The addition of glyphosate and paraquat activated invertase and urease activities in several soils (Sannino and Gianfreda, 2001). Herbicides may disturb C and N cycles, which are very critical for soil quality (Alexander, 1981; Vischetti et al., 2002; Pannacci et al., 2006; Kara et al., 2004; Mahia et al., 2011). Soil microbiological populations may use herbicides and their metabolites as sources of biogenous elements (Cook and Hutter, 1981; Radosevich et al., 1995).

The fate of herbicides in the soil depends on application protocol, dose, physical and chemical properties of soil, humidity, temperature, plant cover, soil cultivation technique, and the soil microorganisms present (Milosevic and Govedarica, 2002). Moisture content, together with temperature, also affects microbial growth and activity in soils (Pietikainen et al., 2005; Uvarov et al., 2006).

The objective of this study was to determine the effects of increasing imazamox doses (RD, 2× RD, and 4× RD) on C and N mineralization of previously imazamox-applied and imazamox-free soils for 45 days [20° C, 60% and 80% of field capacity (FC)].

2. Materials and methods

2.1 Study site and preparation of soil samples

This study was conducted in Osmaniye, 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. Two different plots were selected, 1 without any previous application of imazamox or other pesticides (NI) at Osmaniye Korkut Ata University, and 1 with 6 years of imazamox application (I).

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In August 2011, 5 superficial soil samples were collected from depths of 0–20 cm, 4 from the corners and 1 from the center of both fields. Samples of each field were 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.

2.2. Soil analysis and measurements of the aerobic mineralization

The soil texture was determined with a Bouyoucos hydrometer (Bouyoucos, 1951), field capacity (%) with a vacuum pump at 1/3 atmospheric pressure (Demiralay, 1993), and pH with a WTW Inolab 720 pH-meter in 1:2.5 soil-water suspension (Jackson, 1958). $CaCO_3$ content (%) was measured with a Scheibler calcimeter (Allison and Moodie, 1965), organic carbon content (%) by the Anne method (Duchaufour, 1970), and organic nitrogen content (%) by the Kjeldahl method (Duchaufour, 1970).

Imazamox ($C_{15}H_{19}N_3O_4$; trading name: Raptor Ultra; production firm: Basf; 40 g active ingredient L^{-1}) was added to soil samples at the RD (10 mg kg^{-1}), 2× RD (20 mg kg^{-1}), and 4× RD (40 mg kg^{-1}). Imazamox doses were calculated according to the prospectus (3–4 L ha^{-1}, 40 g active ingredient L^{-1}). The amounts of C and N (mg kg^{-1}) added to the soils via herbicide were 5.88 and 1.38 for RD, 11.8 and 2.76 for 2× RD, and 23.5 and 5.52 for 4× RD, respectively. Soil microbial activity in both of the untreated soils was used as a control.

Soil samples were placed in 750-mL incubation vessels for carbon mineralization and the herbicide dissolved in distilled water was added. The final moisture contents of both soils were adjusted to 60% and 80% of their own FC before incubation at 20 °C over 45 days (Schaefer, 1967). CO_2 derived from microbial activities was absorbed in 40 mL of saturated $Ba(OH)_2$ solution in beakers, placed in the center of the soils in closed incubation vessels, and then transferred to an incubator. The amount of CO_2 produced was measured once every 3 days by titration with oxalic acid (Benlot, 1977). 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.

Nitrogen mineralization of the moisturized soils was observed at 20 °C for 45 days. Soil samples were shaken with 200 mL of 1 N $CaCl_2$ solution for 1 h. They were distilled to measure mineral nitrogen (NH_4 + NO_3) by the Parnas–Wagner method after filtering (Lemée, 1967; Gökçeoğlu, 1979).

2.3. Statistical analysis

Repeated measures (general linear model) analysis was performed to determine the differences in carbon and nitrogen mineralizations over incubation time between the 2 soils, herbicide doses, and different humidities (Kleinbaum et al., 1998). Three replicates were used for each combined soil for statistical comparisons. Data were analyzed by a series of analyses of variance. Differences between data were assumed significant at $P < 0.05$. All statistical analyses were carried out using SPSS 11.5.

3. Results

NI and I soils used in incubation differed in some of their physical and chemical properties (Table 1). Both soil samples were slightly alkaline and sandy loam-textured. The FC of I and NI soils was 19.5% and 34.4%, respectively. There were significant differences between I and NI soils

Table 1. Some physical and chemical properties of NI and I soils.

Characteristics	Sites		Significance between sites
	I	NI	
Sand [2–0.02 mm (%)]	68.3 ± 1.91	72.8 ± 1.88	0.163
Silt [0.02–0.002 mm (%)]	11.5 ± 0.11	14.2 ± 2.58	0.353
Clay [<0.002 mm (%)]	20.3 ± 1.95	13.0 ± 0.70	0.025
Texture type	Sandy loam	Sandy loam	–
Field capacity (%)	19.5 ± 1.02	34.4 ± 0.09	0.000
pH	7.49 ± 0.008	7.97 ± 0.03	0.000
$CaCO_3$ (%)	4.20 ± 0.11	12.9 ± 0.06	0.000
C (%)	1.62 ± 0.03	6.02 ± 0.14	0.000
N (%)	0.14 ± 0.009	0.38 ± 0.03	0.001
C/N ratio	11.9 ± 1.08	16.2 ± 1.48	0.081

Mean ± SE, n = 3.

regarding $CaCO_3$ content (%, $P < 0.001$). Both soil organic carbon content (%) and soil nitrogen content (%) were also statistically significant between the 2 soils ($P < 0.01$). C/N ratios of I and NI soils were 11.9 and 16.2, respectively.

Cumulative $C(CO_2)$ respired increased significantly with incubation time in both NI and I soils (Figures 1 and 2). In this context, the control of I soil humidified with 80% FC was significantly higher than the 4× RD I soil, as well as the 2× and 4× NI soils humidified with 60% FC ($P < 0.05$). The RD of imazamox in I soil was statistically different from 2× and 4× RD in I soil at 80% humidity, which was also different from 2× and 4× RD in I soil and RD, 2×, and 4× RD in NI soil at 60% humidity ($P < 0.05$). The control of NI soil was significantly higher than 4× RD in I soil at 80% FC and 2× and 4× RD in NI soil at 60% FC ($P < 0.05$). Cumulative $C(CO_2)$ of the control and RD in I soil were significantly different from 4× RD ($P < 0.05$) at 60% humidity. The control and RD of I soil were also different from 2× RD ($P < 0.05$) and 4× RD ($P < 0.05$) of NI soil at this humidity. The carbon mineralization rate (%) of the control and RD in I soil was statistically higher than 2× and 4× RD ($P < 0.05$, Figure 3). This rate was significantly lower in NI soil than in I soil at all doses of imazamox and at both humidities ($P < 0.05$).

NH_4-N and NO_3-N contents (mg kg^{-1}) are given in Table 2. NH_4-N content was not affected by herbicide and soil type at either humidity ($P > 0.05$). NO_3-N production in both I and NI soils decreased regularly with increasing doses of the herbicide at both humidities ($P < 0.05$) (Table 2). The N mineralization rate was significantly different between control and the imazamox doses in I and NI soils at both 80% and 60% of FC ($P < 0.05$).

4. Discussion

The highest carbon mineralization was observed in the control of I soil humidified to 80% FC, which might be the most suitable humidity for soil microorganisms (Figures 1 and 2). Microorganisms in carob and acacia soils also showed better activities under 80% FC at 28 °C over 30 days (Zengin et al., 2008a, 2008b). The RD of imazamox in I soil at 80% humidity showed a higher microbial activity than the other herbicide doses at both humidities. This dose may be suggested as the most favorable for soil microorganisms at 80% FC. Similar results in carbon

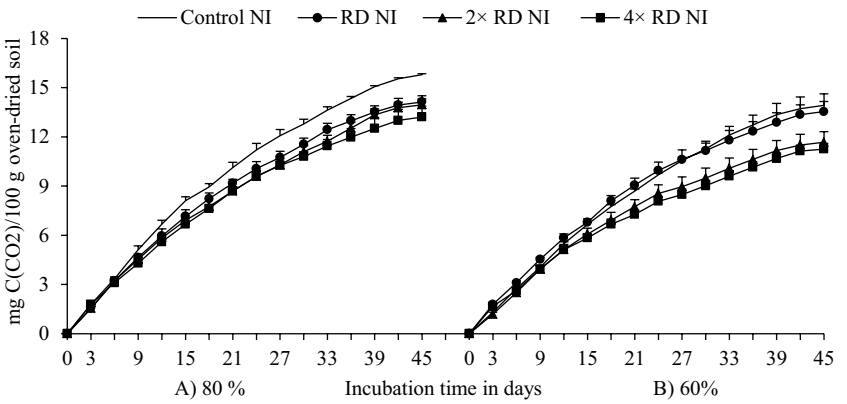

Figure 1. Cumulative carbon mineralized (mean ± SE, n = 3) at 2 levels of humidity, (A) 80% and (B) 60%, of NI soil at RD, 2×, and 4× RD of imazamox over 45 days at 20 °C.

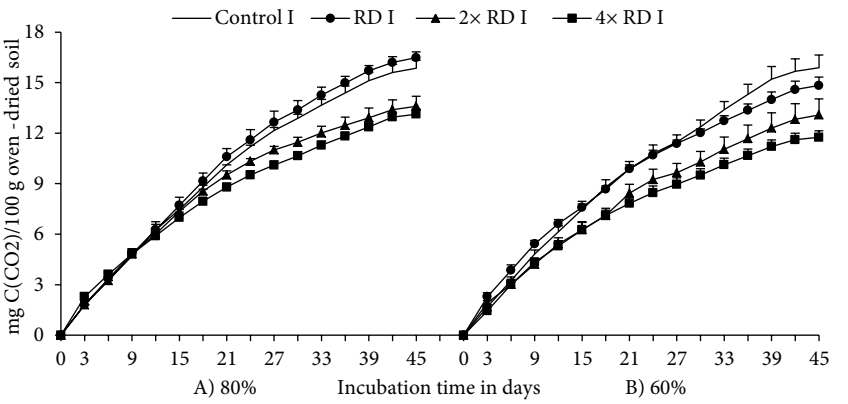

Figure 2. Cumulative carbon mineralized (mean ± SE, n = 3) at 2 levels of humidity, (A) 80% and (B) 60%, of I soil at RD, 2×, and 4× of RD of imazamox during 45 days at 20 °C.

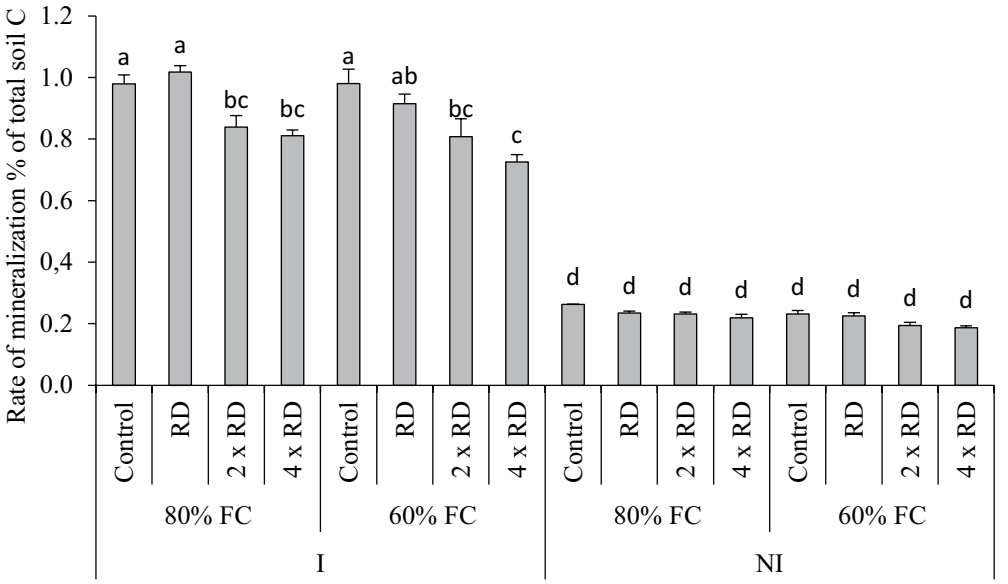

Figure 3. Rate of carbon mineralization in the control of NI and I soils at RD, 2×, and 4× of RD of imazamox at 2 levels of humidity (mean ± SE, n = 3) during 45 days at 20 °C. Different letters denote significant differences among 2 fields, 2 levels of humidity, and different doses of imazamox ($P < 0.05$).

Table 2. Nitrogen mineralization (mg kg^{-1}) of NI and I (before and after carbon mineralization, 45 days and 20 °C).

			NH_4-N (mg kg^{-1})			NO_3-N (mg kg^{-1})			N mineralization rate (%)		
Before C mineralization			5.16 ±	0.33	bc	3.67 ±	0.31	i	0.52 ±	0.01	h
I	80 % FC	Control	5.86 ±	0.33	bc	21.0 ±	2.01	ef	1.58 ±	0.11	a
		RD	4.28 ±	0.78	c	11.0 ±	0.71	ghi	0.90 ±	0.01	efg
		2× RD	7.08 ±	0.79	abc	7.29 ±	0.68	hi	0.85 ±	0.01	efg
		4× RD	8.36 ±	0.43	ab	6.91 ±	0.30	hi	0.90 ±	0.04	efg
	60 % FC	Control	5.87 ±	0.32	bc	19.1 ±	0.76	efg	1.47 ±	0.03	ab
		RD	5.65 ±	0.14	bc	12.2 ±	0.33	gh	1.05 ±	0.02	cde
		2× RD	5.92 ±	0.53	bc	6.38 ±	0.22	hi	0.72 ±	0.04	fgh
		4× RD	5.57 ±	0.42	bc	3.23 ±	0.76	i	0.52 ±	0.06	h
Before C mineralization			5.07 ±	0.40	bc	8.81 ±	0.30	hi	0.37 ±	0.01	h
NI	80 % FC	Control	7.11 ±	0.39	abc	53.5 ±	3.14	a	1.60 ±	0.08	a
		RD	5.52 ±	0.07	bc	43.1 ±	3.97	b	1.28 ±	0.10	bc
		2× RD	5.94 ±	1.05	bc	34.6 ±	0.52	cd	1.07 ±	0.02	cde
		4× RD	6.36 ±	0.56	abc	22.9 ±	0.67	ef	0.77 ±	0.01	fgh
	60 % FC	Control	7.35 ±	0.69	abc	37.7 ±	2.17	bc	1.18 ±	0.06	cde
		RD	9.35 ±	0.17	a	27.2 ±	0.76	de	0.96 ±	0.02	def
		2× RD	5.25 ±	0.42	bc	23.7 ±	1.43	ef	0.76 ±	0.05	fgh
		4× RD	7.13 ±	1.17	abc	17.0 ±	0.57	fg	0.63 ±	0.03	gh

Mean ± SE, n = 3, FC: field capacity, RD: recommended dose. Different letters (a, b, c, d, e, f, g, h, and i) denote significant differences ($P \leq 0.05$) among 2 fields, 2 humidity levels, and different dose levels of the herbicide imazamox.

mineralization were also observed in herbicide-added soils, such as imazamox and benfluralin (Vischetti et al. 2002), glyphosate isopropylamine and trifluralin (Eser et al., 2007), trifluralin (Aka Sağlıker, 2009), and atrazine (Mahia et al., 2011). Carbon mineralization in both 2× and 4× RD in I and NI soils was significantly lower than that in the control and RD in I soil at 60% humidity. This might be explained by the lower humidity and higher dose of imazamox at 20 °C. It was shown that increased moisture and temperature accelerate the degradation of atrazine and 2,4-D (Willems et al., 1996). The mineralization rate with atrazine may be ignorable (<2%) at low temperatures and moistures (Milosevic and Govedarica, 2002).

Carbon mineralization rates (%) at all herbicide doses and both humidities in NI were significantly lower than that of I soil. It is possible to conclude that some soil microorganisms are able to consume imazamox as carbon and nitrogen sources in I soil. Similar results were also observed with different herbicide-added soils (Kara et al., 2004; Eser et al., 2007; Aka Sağlıker, 2009; Mahia et al., 2011). Pannacci et al. (2006) found that imazamox availability was greatest in sandy soils and decreased in soils with high organic carbon content. The current study results confirm this through differences between cumulative carbon mineralizations of both soils.

NH_4-N content (mg kg^{-1}) was dependent neither on soil type nor on herbicide dose, which might be due to the high ecological potential of ammonia bacteria in the soil. Pesticides can stimulate or inhibit N mineralization (Chen et al., 2001; Haney et al., 2002). It was estimated that pesticides applied at recommended doses had no long-term harmful effects on soil microbial activity as assessed by N mineralization (Hart and Brookes, 1997). Mahia et al. (2011) found that nitrogen mineralization increased significantly with the incubation time in atrazine-added soils. They found nonpersistent effects of atrazine addition on N mineralization at longer incubation times (9–12 weeks). Similarly, nitrate production at all herbicide doses in I soil was significantly lower than in NI soil at both humidities in the present study. These results showed that imazamox addition to these soils significantly decreased the activities of nitrate-producing bacteria. Edwards (1989) also confirmed that nitrifying bacteria are most sensitive to herbicide application. This may indicate the sensitivity of these microorganisms to foreign compounds within the frames of soil ecological equilibrium.

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