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Article

Impact of Herbicide Oxadiazon on Microbial Activity and Nitrogen Dynamics in Soil Environment

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Influence of herbicide oxadiazon on soil microbial activity and nitrogen dynamics was evaluated. Soil samples were treated with oxadiazon at field and tenfold field rates and incubated. Organic amendment was added as an additional substrate for soil microorganisms. Tenfold field rate oxadiazon stimulated substrate-induced respiration (SIR) and dehydrogenase activity (DHA) in amended soil as compared to unamended soil and control treatment. Soil urease activity was not affected by oxadiazon treatment. In both amended and unamended soils, treatment of the herbicide at higher rate had not significant influence on NH_4-N and NO_3-N concentrations. Higher dose of oxadiazon was degraded in both soils, but dissipation rate in amended soil was higher than unamended soil, with half-lives ($t_{1/2}$) of 23.1 and 138.6 days, respectively. Recommended field rate did not affect microbial activity and nitrogen dynamics in soil ecosystem. Results showed influence of oxadiazon on cycling processes of nitrogen in soil was not significant however its effect on microbial activity was a tendency depending on addition of organic amendment to soil.

Key words: *Herbicide oxadiazon, soil microbial activity, nitrogen dynamics.*

Oxadiazon [5-*tert*-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3*H*)-one] is incorporated as a pre-emergent soil-applied herbicide into the agro-ecosystem for controlling annual grasses and broadleaf weeds in rice, cotton, onion, potato, soybeans, peanut, and ornamentals. Although the herbicide may have a beneficial impact on the agricultural productivity, nonetheless, environmental impact of this organic chemical when applied intentionally to soil ecosystem is of much concern. The addition of this xenobiotic organic substance may affect species composition and abundance of soil microorganisms, enhancement or suppression of enzyme production, and overall activity of enzyme in soil.[1] Upon application, a large portion of this herbicide accumulates in the soil system, where most of the soil ecological activities occur.[2,3] The presence of herbicides in soil may cause changes in the microbial population and activity of soil, and microbial degradation is considered to be the most important transformation processes determining the persistence of herbicides in soil.[4]

The overall enzyme activity of soil is a composite of various enzyme components produced by the microorganisms in soil. The enzymes in soil most frequently studied are oxidoreductases such as dehydrogenase and hydrolases such as urease. Therefore, measurement of enzyme activity in soil can be used as a possible tool for determining the biological activity in soil, which may be used as an index of soil fertility.[5] Due to the important roles of soil enzymes in nutrient cycling and soil fertility, enzyme studies have introduced guidelines on assessing the effect of pesticides on soil microflora. in subsequent studies on the environmental impacts of herbicides, soil activities of many enzymes have been measured.[6,7] The impact of these agrochemicals on soil enzyme activities depend on the chemical nature and dose of the herbicide, the type of enzyme and soil, and the experimental condition.

In the mineralization cycle of nitrogen, ammonification and nitrification processes in soil provide nitrogen as a plant nutrient in the utilizable form. In the soil system, microbial conversion of ammonium into nitrate is catalyzed by the nitrifying bacteria. These chemoautotrophic bacteria have a major role in the oxidation of NH_4^+ and grow in the absence of organic C. Therefore, microorganisms are of prime importance in the cycling of key element essential for biological processes and thus for the maintenance of soil fertility. Concern on the effects of pesticides in the biological processes is increasing, because the pesticides have the potential to be active in all organisms. Studies of pesticide effects on the microbial parameters in relation to the nitrogen cycle revealed that pesticides appeared to have variable impacts on the mineralization process.[8,9]

Only few studies have been reported the effect of herbicidal on soil microorganisms and nitrogen mineralization. Therefore, the objectives of this study were to assess the effect of herbicide oxadiazon on the microbial activities in soil and to evaluate the influence of organic amendment on soil microbial activities in response to the applied herbicide.

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Materials and Methods

Agrochemicals and chemicals. The herbicide oxadiazon (99.95% purity) was provided by Rhone-Poulenc, Agro-Division (France). Reagent grade urea was purchased from Yakuri Pure Chemicals Co. (Osaka, Japan). All other chemicals were of reagent grade supplied by Sigma Chemical Co. (St. Louis, MO, USA), Junsei Chemical Co. (Tokyo, Japan) and Kanto Chemical Co (Tokyo, Japan).

Soil and organic amendment. Surface soil sample (0-20 cm) was collected from a pesticide-free experimental field of Kyungpook National University, Daegu, Korea. The soil was clay loam (33.2% clay, 42.8% silt, and 24.0% sand) with 1.0% organic matter, 13.2 cmol/kg CEC, and pH (H_2O) 7.3. Organic amendment with finely ground and sieved after purchasing commercial product (Npko®, Korea) was added to a portion of the soil at approximately 50 : 1 (soil : amendment, dry wt. basis) before application of solution, and the soil samples were mixed thoroughly with the organic amendment (amended soil).

Experimental treatment. Prior to the initiation of experiment, soil samples were pre-incubated for 2 weeks after the addition of organic amendment. Urea fertilizer solution was added to the soil samples at 10 mg/100 g soil. In addition to the fertilizer treatment, a fine spray of oxadiazon was applied at approximate field rate (1.0 mg a.i./kg soil) and tenfold field rate (10.0 mg a.i./kg soil) onto the soil samples. The soil moisture was about 40% (dry wt. basis) after the spraying, and water was added once or twice a week to maintain the soil moisture content between 30 and 40% (dry wt. basis). The control soils received equal amount of deionized water. All treated soils including the control were incubated at 28 ± 1°C for 56 days. After 3, 7, 14, 28, and 56 days, the soil samples were processed and analyzed.

Analytical methods. Soil microbial activity was assessed to measure the substrate-induced respiration (SIR) and activity of soil enzymes such as dehydrogenase and urease. SIR was measured as the rate of CO_2 evolution during 6 h incubation after the addition of 5 m*l* glucose solution (32 mg/m*l*) to the soil. The evolved CO_2 was trapped with 0.02 N NaOH, precipitated with 3 N $BaCl_2$, and titrated with 0.02 N HCl using an autotitrator (Metrohm 702 SM Titrino, Herisau, Switzerland) as cited by Anderson and Domsch.[10] Dehydrogenase activity (DHA) of soil was determined as the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) into 1,3,5-triphenyl formazan (TPF) using UV-Spectrophotometer (Hitachi, Tokyo, Japan) following the method of Tabatabai.[11] Soil urease activity was also determined colorimetrically using UV-Spectrophotometer (Hitachi, Tokyo, Japan) as mentioned by Tabatabai and Bremner.[12] Extractable soil inorganic N concentrations (NH_4-N and NO_3-N) were analyzed by ion chromatograph (IC) equipped with a conductivity detector (Sykam, Eresing, Germany) using 10 mM KCl as the soil extractant.[13] Analytical conditions of IC for anion and cation analyses were respectively as follows: separation columns, 3 × 250 mm Sykam LCA A14 and 125 × 4.6 mm Sykam LCA SS; temperatures, 70 and 40°C; elution systems, 7.5 mM Na_2CO_3 and 0.2% modifier (4-hydroxy benzonitrile in methanol), and 0.61 mM ascorbic acid and 3.27 mM oxalic acid; flow rates, 1.50 and 1.00 m*l*/min; and injection volumes, 50 and 10 m*l*. Soil residue analysis of oxadiazon was performed by gas chromatograph (GC) equipped with an electron capture detector (Varian 3800X, Walnut Creek, CA, USA) using acetone as the soil extractant.[14] Analytical conditions for GC were as follows: column, 30 m × 0.25 mm i.d. capillary column DB-1; respective temperatures of column oven and detector block, 240 and 300°C; N_2 carrier gas flow rate, 1.0 m*l*/min; and injection volume, 1.0 m*l*.

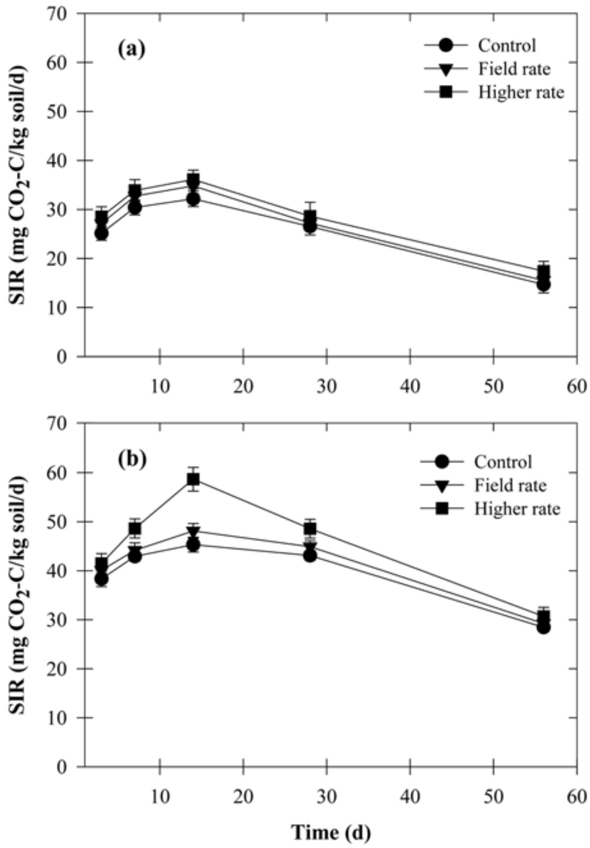

Fig. 1. Substrate induced respiration of the unamended (a) and amended (b) soils treated with oxadiazon. Error bar represents standard deviation of mean.

Results and Discussion

Effect of oxadiazon on soil microbial activities. Both SIR and DHA are commonly used as indices of the overall microbial activity of the soil ecosystem. In this study, the microbial activity was measured in terms of soil respiration based on the evolution of CO_2. Oxadiazon application had no significant influence on the SIR rate in the unamended soil (Fig. 1). In the amended soil, the field rate of oxdiazon application had no significant influence on SIR rate throughout the experimental period. However, at higher rate of oxadiazon application, SIR started to increase at early of

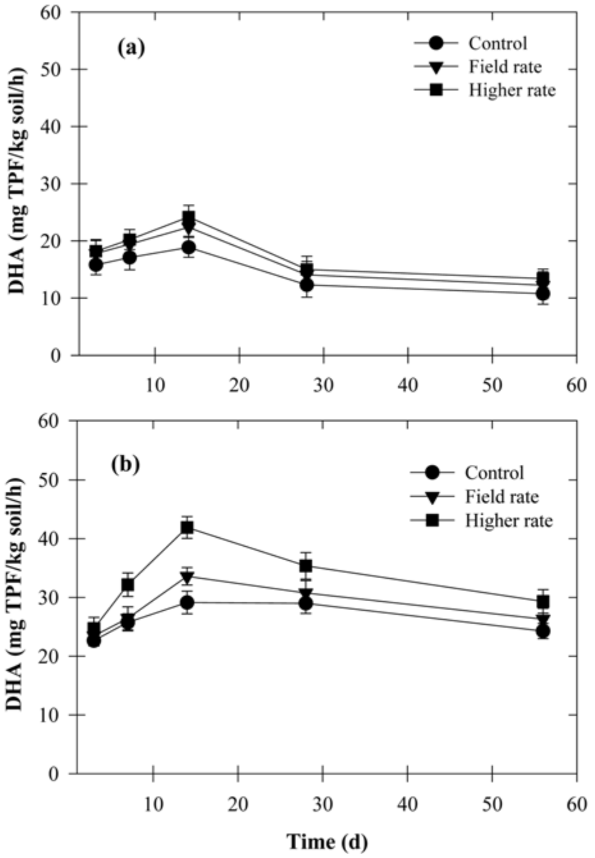

Fig. 2. Dehydrogenase activity of the unamended (a) and amended (b) soils treated with oxadiazon. Error bar represents standard deviation of mean.

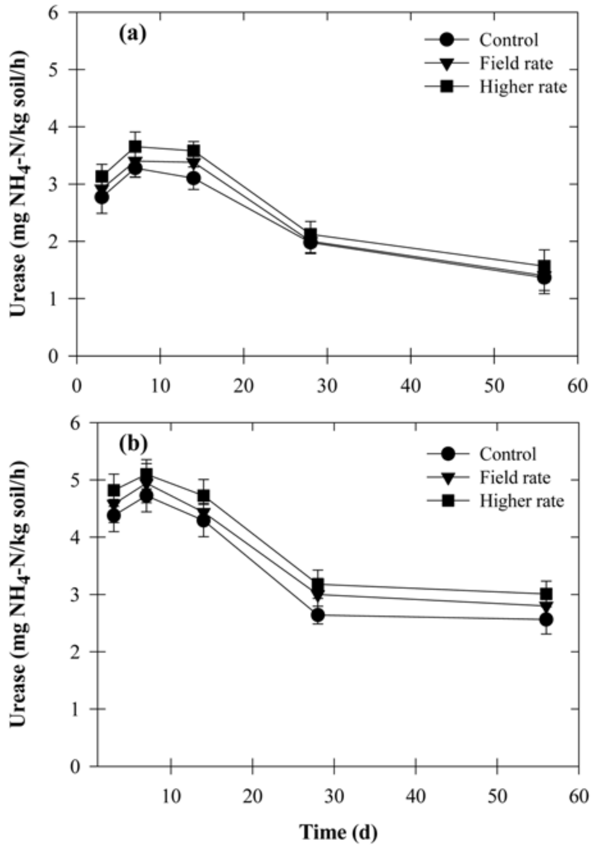

Fig. 3. Urease activity of the unamended (a) and amended (b) soils treated with oxadiazon. Error bar represents standard deviation of mean.

incubation, and then underwent little or no effect toward the end of incubation (Fig. 1). Although we did not quantify the number of soil microorganisms, the stimulation of SIR could be an indirect indication of the influence of herbicide treatment on the soil microbial populations. Araujo *et al.*[4] and Haney *et al.*[15] assessed the impact of herbicide glyphosate on the microbial community of soils and also found that the microbial activity was enhanced in the presence of the herbicide. In this experiment, SIR was comparatively higher in the amended soil than the unamended soil, as similarly found in 10 other different soils.[16] In the unamended soil, the application of oxadiazon had no significant influence on DHA in comparison to the control treatment (Fig. 2). Soil dehydrogenase activity increased up to 14 days in response to the higher application rate of the herbicide, then decreased thereafter in the amended soil (Fig. 2), possibly because the herbicide stimulated certain groups of microorganisms, leading to increased soil microbial population.[17] The presence of crop residues as organic amendment stimulated not only the size of microbial population but also the microbial activity.[18] Therefore, the applied herbicide could serve as a substrate for the population increase of microbial species. The interaction between pesticide and dehydrogenase suggest microbial activity could be a useful indicator for testing the effect of agrochemicals.[19] Davies and Greaves[20] reported that stimulation of DHA by some herbicides was due to the microbial utilization of either the chemical itself as a carbon source or microorganisms killed by the chemical. In both soils, urease activity was not affected by oxadiazon treatment throughout the incubation period (Fig. 3). Davies et. al. and Schinner et. al. also demonstrated that urease activity was not affected by some herbicide.[20,21] Urease activity by treatment of organic amendment was comparatively higher in the amended soil than the unamended soil. Microorganisms are predominantly responsible for soil urease activity, which is correlated with the soil organic matter content.[22] However, at high concentrations the stimulative effect of oxadiazon on soil urease activity could be the result of reactivities between oxadiazon and urease resulting from changes in the number or activity of microorganisms caused by the addition of organic amendment as an additional substrate for soil microorganisms. This, in turn, caused the altered levels of enzymes in the soil ecosystem.[1] On the contrary, oxadiazon had no influence on the microbial activity when applied at the recommended field rate. At normal field application levels, pesticides generally do not exhibit significant effects (both stimulation and inhibition) on the soil microbial activities.[23,24]

Effect of oxadiazon on soil nitrogen dynamics. Ammonification and nitrification, part of the nitrogen mineralization cycle in soil, provide nitrogen for plant growth.

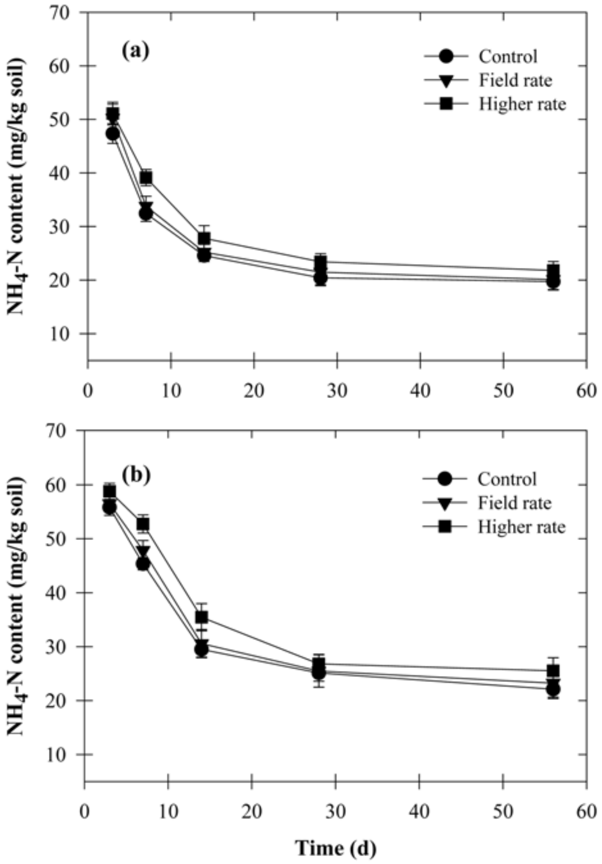

Fig. 4. Ammonium content of the unamended (a) and amended (b) soils treated with oxadiazon. Error bar represents standard deviation of mean.

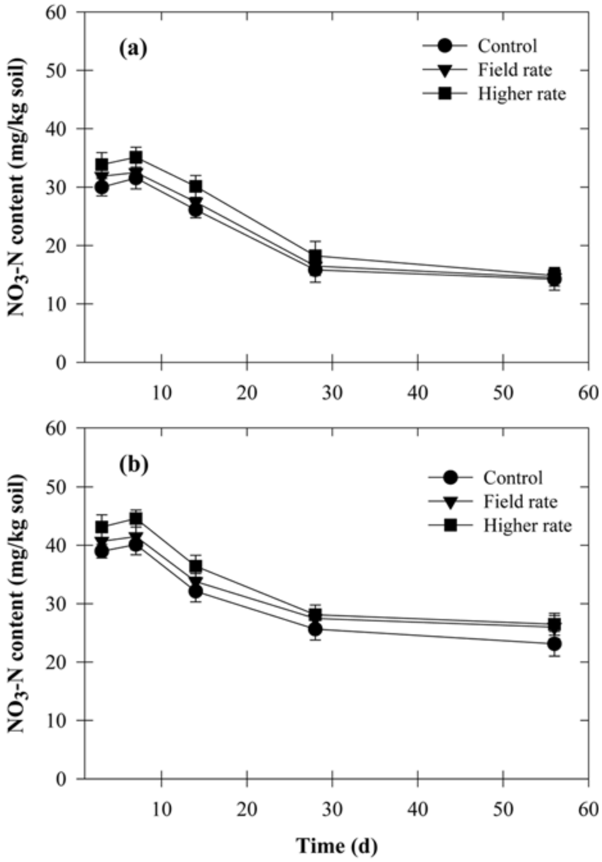

Fig. 5. Nitrate content of the unamended (a) and amended (b) soils treated with oxadiazon. Error bar represents standard deviation of mean.

The results illustrated that oxadiazon treatment had no significant influence on NH_4-N content in both soils (Fig. 4). In addition, no significant effects were observed on NO_3-N concentrations of both unamended and amended soils treated with higher doses of this herbicide (Fig. 5). During the mineralization processes, the nitrifying bacteria responsible for oxidation of ammonium into nitrate in the soil system are chemolithotrophs, which derive their energy from the oxidation of reduced inorganic substances.[25] These chemolithotrophic bacteria relied on a biochemical reaction (oxidation) by the means of using oxygen as a way of transporting electrons drawn from the ammonia and nitrite compounds but did not depend on this herbicide as an energy source. Chen *et al.*[8] studied the effect of the herbicide butachlor on nitrogen transformation using similar rates and found that the higher dose did not affect ammonification and nitrification processes in the soil system. This finding showed that this herbicide has no marked side effect on the nitrifying bacteria in the soil ecosystem.

Persistence of oxadiazon residue in soil system. The persistence of the herbicide oxadiazon varied in both the unamended and amended soils (Fig. 6). In the soil system, the dissipation of higher rate of oxadiazon in the amended soil was more rapid after application than the unamended soil, whereas the dissipation trend of the field rate was not remarkable

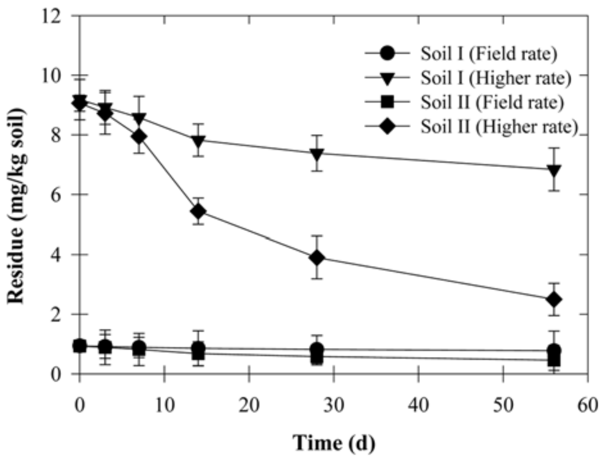

Fig. 6. Degradation of oxadiazon in the unamended (soil I) and amended (soil II) soils. Error bar represents standard deviation of mean.

in both soils. Some studies cited similar persistence of oxadiazon in soil.[26,27] The half-lives ($t_{1/2}$) of oxadiazon in unamended and amended soils were 138.6 and 23.1 days, respectively as determined through the first-order reaction kinetics.[28] The accelerated degradation due to microbial adaptation caused shortened half-life and loss of efficacy.[29] Dzantor and Felsot[30] stated that the enhanced degradation of herbicides might be due to the accumulation of the organic matters caused by the overall stimulation of microbial activity. In addition, organic amendments applied to increase the low organic matter content promoted the herbicide adsorption in soil.[31] In this investigation, a stimulating effect of oxadiazon was observed on soil microbial activity in the amended soil, but its impacts on soil nitrogen dynamics was not significant.

In summary, we have shown that oxadiazon treated with field application rate did not show any effect on the microbial activities and nitrogen dynamics in soil regardless of the addition of organic amendment.

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