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## Effect of Endosulfan, Deltamethrin and Profenophos on Soil Microbial Respiration Characteristics in Two Land Uses Systems in Burkina Faso

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**Abstract:** The effect of endosulfan, deltamethrin and profenophos on microbial respiration parameters were studied in soil from cultivated and fallow plot from Burkina Faso. Microcosms of soil were incubated at 25.0°C. All pesticides were applied separately at the rate of 200 mg kg<sup>-1</sup> and then 7 days later, glucose, nitrogen and phosphorus were added to the incubated soil. Basal respiration was higher with cultivated plot (0.034±0.015 mg-CO<sub>2</sub> g<sup>-1</sup> soil) compared with fallow plot (0.028±0.014 mg-CO<sub>2</sub> g<sup>-1</sup> soil). With cultivated soil, endosulfan application decreased Basal Respiration while an increase was observed with profenophos and deltamethrin applications and the control. Substrate Induced Respiration varied from 0.014 to 0.016 mg-CO<sub>2</sub> g<sup>-1</sup> soil and 0.026 to 0.034 mg-CO<sub>2</sub> g<sup>-1</sup> soil in cultivated soil and fallow soil, respectively. Short lag time (11-14 h) was observed with cultivated soil as compared with soil from fallow plot (53-69 h). Pesticide application did not affect significantly the lag time with cultivated soil, while with fallow soil, significant reduction were observed. In the two soils, pesticide application anticipated the apparition of the maximum respiration rate compared to the control.

**Key words:** Pesticides, basal-respiration, substrate-induced-respiration, lag-time

### INTRODUCTION

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9a-hexahydro-6, 9-methano-2, 3, 4-benzodioxanthiepin-3-oxyde), deltamethrin [(S)- $\alpha$ -cyano-3-phenoxybenzyl (1R)-cis-3-(2, 2-dibromovinyl)-2, 2-dimethyl-cyclopropanecarboxylate] and profenophos (O-4-bromo-2-chlorophenyl-O-ethyl-S propyl phosphorothioate) are insecticides widely used in cotton, cereal and vegetable fields in Burkina Faso. Improper use of pesticides combined with their persistence, volatilization and mobility lead to frequent detections of the pesticide residues in the atmosphere, soil, surface water and in vegetable fruits and cereal grains. Persistence of endosulfan, profenophos and other organo-chlorinated pesticides (dieldrine, endrine) in the cotton fields in Burkina Faso were reported by Savadogo *et al.* (2006). Others studies across Africa reported 10 years persistence of dieldrin in Uganda (Déjoux, 1988) and contamination of soil, water and sediments by DDT, HCH and dieldrin in Tanzania (Kishimba *et al.*, 2004).

Results from previous studies on the effects of pesticides on soil microbial activity were contradictory. These findings indicated a decrease in soil microorganism biomass (Lin and Brookes, 1999; Hussain *et al.*, 2001), disturbance in soil microorganism respiration

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(Pozo *et al.*, 1995; Savadogo *et al.*, 2009), or depressive effects on enzymatic activity (Mokiedje and Spiteller, 2002) and microbial diversity (Johnsen *et al.*, 2001; Katayama *et al.*, 2001). On the other hand, glyphosate applied at the rate 234 mg kg<sup>-1</sup> of soil stimulated soil microorganism biomass and activity measured as carbon and nitrogen mineralisation (Haney and Senseman, 2000; Haney *et al.*, 2002; Araújo *et al.*, 2003). Application of 2, 4-D, picloram and glyphosate both at the rate of 200 mg kg<sup>-1</sup> increase basal respiration (Wardle and Parkinson, 1990). In sandy soil, Substrate Induced Respiration (SIR) increases following application of tebuconazole or cyhalothrin (Cycón *et al.*, 2006). However, several studies reported no effect of pesticides on soil microorganisms' respiration (Zelles *et al.*, 1985; Haney and Senseman, 2000; Savadogo *et al.*, 2009).

Soil physical and chemical characteristics such as structure, texture, pH, temperature, organic matter content and water holding capacity determine the fate of pesticides. Soils in semi-arid Africa including Burkina Faso are highly weathered with low organic matter and nutrient content (Bationo *et al.*, 1998), which may lead to different fate of pesticide compare with temperate soil having different biotic and abiotic conditions. Studies on pesticide effect on soil microorganisms' respiration are scarce in semi-arid Africa.

The aim of this study was to assess the effects of endosulfan, deltamethrin and profenophos on soil microorganisms' respiration characteristics. We hypothesised that, repeated application of pesticide in the field inhibits soil microbial activity, leading to a decrease in respiratory activity and microbial biomass.

## **MATERIALS AND METHODS**

### **Study Site**

The study was carried out from June to September 2008 in cultivated (vegetable) fields located around the main dam of Ouagadougou (12°27'; 1°30' and 300 above the sea level) in Burkina Faso, West Africa. Soils in this area are mainly silty-sandy and clay-sandy. Four farmers (replicate) having similar cultural practices were selected. These farmers applied high rate of organic matter, mineral fertilizers and pesticides. In the vicinity of each selected field, one fallow plot which had never received any application of pesticides and organic matter was selected as control plot.

### **Soil Sampling**

Composite soil samples from five spots at 0-20 cm were taken from each plot in July 2008 using an auger (6 cm diameter). Samples were sieved through 2 mm mesh for respiration tests and chemical determinations.

### **Soil Chemical Determination**

The soil pH(H<sub>2</sub>O) and pH(KCl) were determined using an electronic pH-meter in 1 g soil to 2.5 mL water and KCl 1 M slurries, respectively. Total soil carbon content was determined using Walkley and Black method (Walkley and Black, 1934).

### **Experimental Design and Layout**

To evaluate the effect of pesticides on soil microorganisms' activity, we used a factorial design with two main factors that were replicated four times:

Land use system with two levels: (1) cultivated soil (soil with highest OM content) and (2) fallow soil (with lowest organic matter).

Type of pesticide with four levels: (1) no pesticide application, (2) application of endosulfan, (3) application of deltamethrin and (4) application of profenophos. All pesticides were applied during the incubation at the rate of 200 mg kg<sup>-1</sup> of soil.

### Soil Respiration Measurement

Soil respiration rates were determined in 20 g samples of soil humidified at 2/3 of field water holding capacity and incubated at 25.0°C (± 0.5°C) in vessels. The vessels contained 20 mL of sodium hydroxide (NaOH) 0.1 N to trap evolved CO<sub>2</sub> and a small bottle of distilled water to maintain the humidity in the vessel. A reference vessel with only NaOH and distilled water was placed in the same conditions to take into account the initial carbonization of NaOH. The evolved CO<sub>2</sub> and trapped in NaOH was measured titrimetrically, with hydrochloric acid (HCl) 0.1 N. At the time of dosage, 3 mL of barium chloride (BaCl<sub>2</sub>) were added to the NaOH solution to hurl down the CO<sub>2</sub> trapped. The NaOH solution was renewed after every dosage.

### Analysis of Respiration Curves

Models described in previous works (Nordgren, 1992; Schnürer, 2006; Gnankambary *et al.*, 2007) were adapted and used in this study (Fig. 1).

The soil was incubated during 7 days to stabilize respiration rate. The Basal Respiration (BR) was calculated by averaging the 5 daily measurements prior to the pesticide application. After pesticide application, soils were incubated for 7 days to stabilize respiration rate and referred to as BR-pesticide. The Substrate Induced Respiration (SIR) was initiated by adding 0.3 g of glucose, 49.0 mg of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 7.5 mg of KH<sub>2</sub>PO<sub>4</sub>. The SIR reflects microbial biomass and was calculated according to the method described by Anderson and Domsch (1978). The respiration rate remains constant (lag time) for different period depending to the soil and the pesticide, but increase again due to microbial growth. To determine the lag time, evolved CO<sub>2</sub> was measured every 3 h during 72 h. The respiratory quotient (Q) was calculated as BR and SIR ratio.

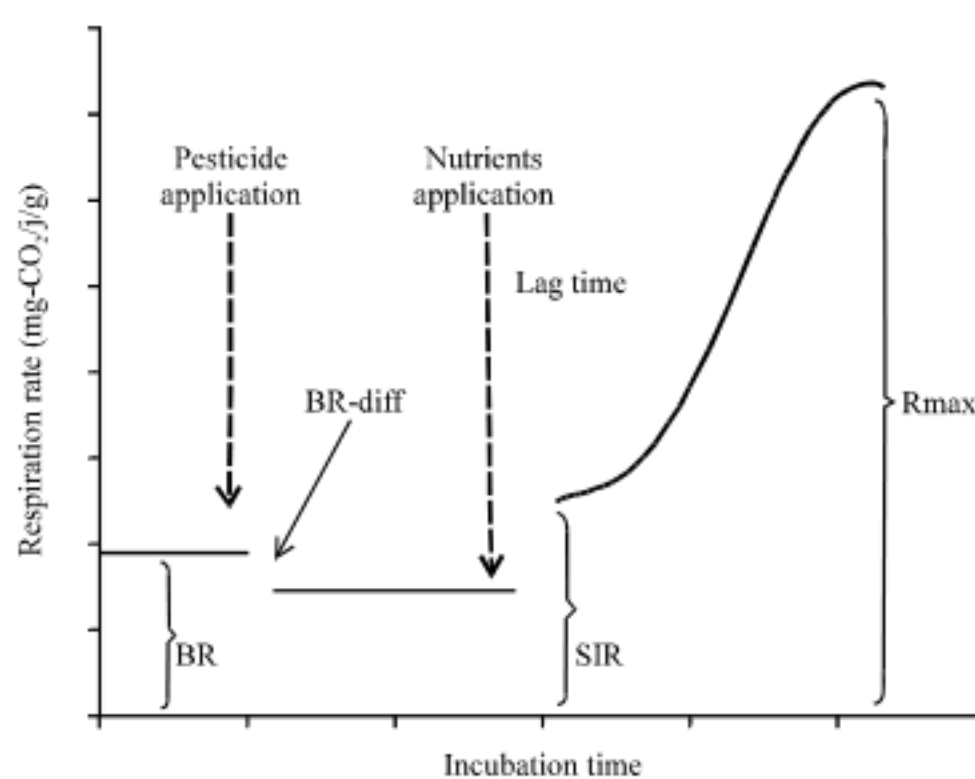


Fig. 1: A model of soil microbial respiration kinetics before and after pesticide and substrate additions. The basal respiration before pesticide addition (BR), the difference between basal respiration after pesticide addition and before pesticide addition (BR-diff), Substrate Induce Respiration (SIR) and maximum respiration rates (Rmax)

### Statistical Analysis

Analysis of Variance (ANOVA) was performed using a General Linear Model (GLM) implemented in Minitab (V. 14) statistical software for Windows (Minitab Inc.). Differences were significant when  $p < 0.05$  according to Tukey's tests (Tukey, 1977).

## RESULTS

Cultivated soil had higher OM content than fallow soil. With the cultivated soil, organic matter content (%), pH (water) and pH (KCl) were  $1.5 \pm 0.01$ ;  $8.4 \pm 0.06$ , and  $7.8 \pm 0.09$ , respectively. The values were  $0.9 \pm 0.01$ ;  $8.03 \pm 0.06$ ; and  $8.03 \pm 0.05$ , respectively, with soil from fallow plot.

General patterns of soil microbial respiration rates were showed in Fig. 2a and b. Basal respiration tended to be higher with cultivated plot ( $0.034 \text{ mg-CO}_2 \text{ g}^{-1} \text{ soil}$ ) compared with fallow plot ( $0.029 \pm 0.014 \text{ mg-CO}_2 \text{ g}^{-1}$ ).

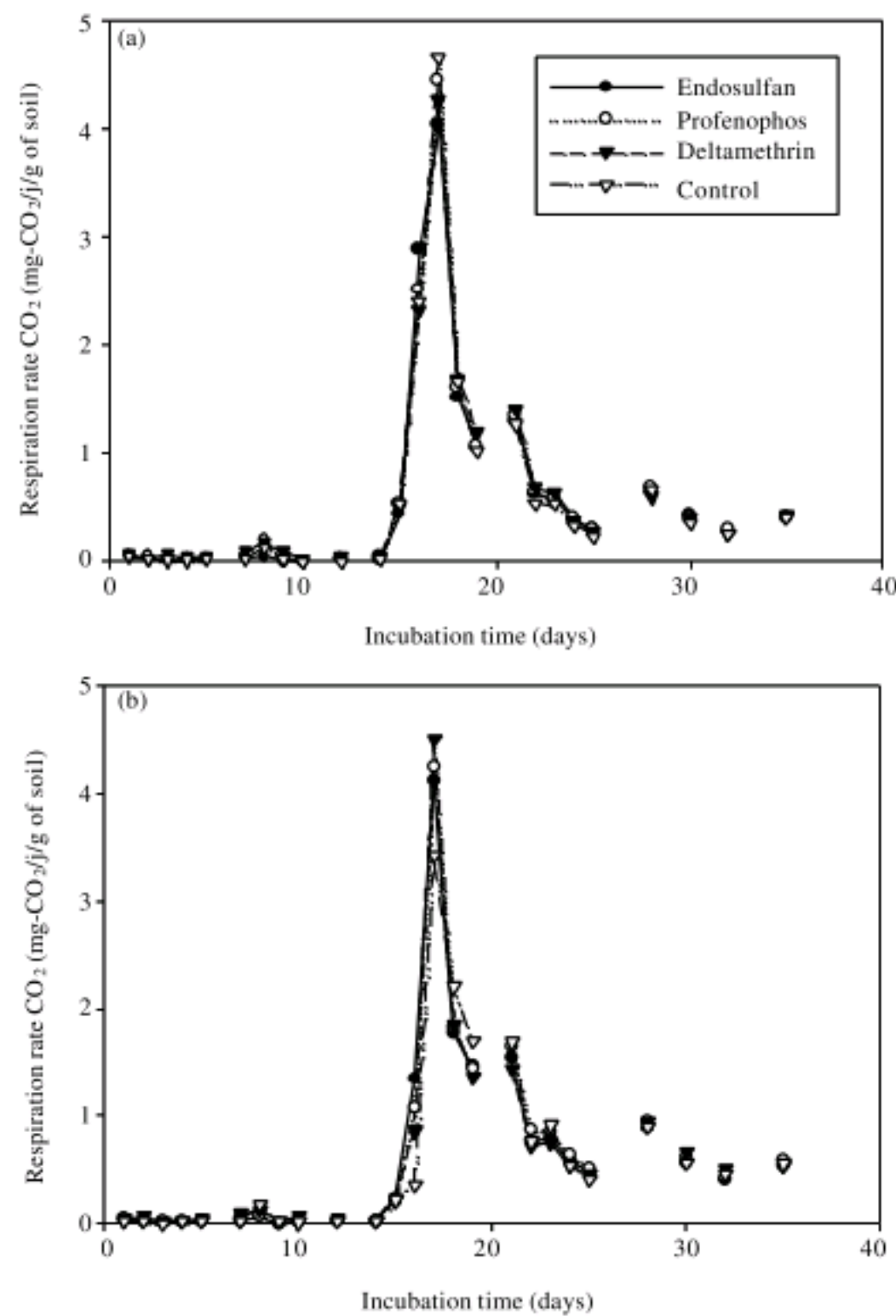


Fig. 2: (a) Microbial respiration kinetics of soils from cultivated plots and (b) fallow plot before and after addition of pesticides and nutrients. Each point represents an average for the four field replicates

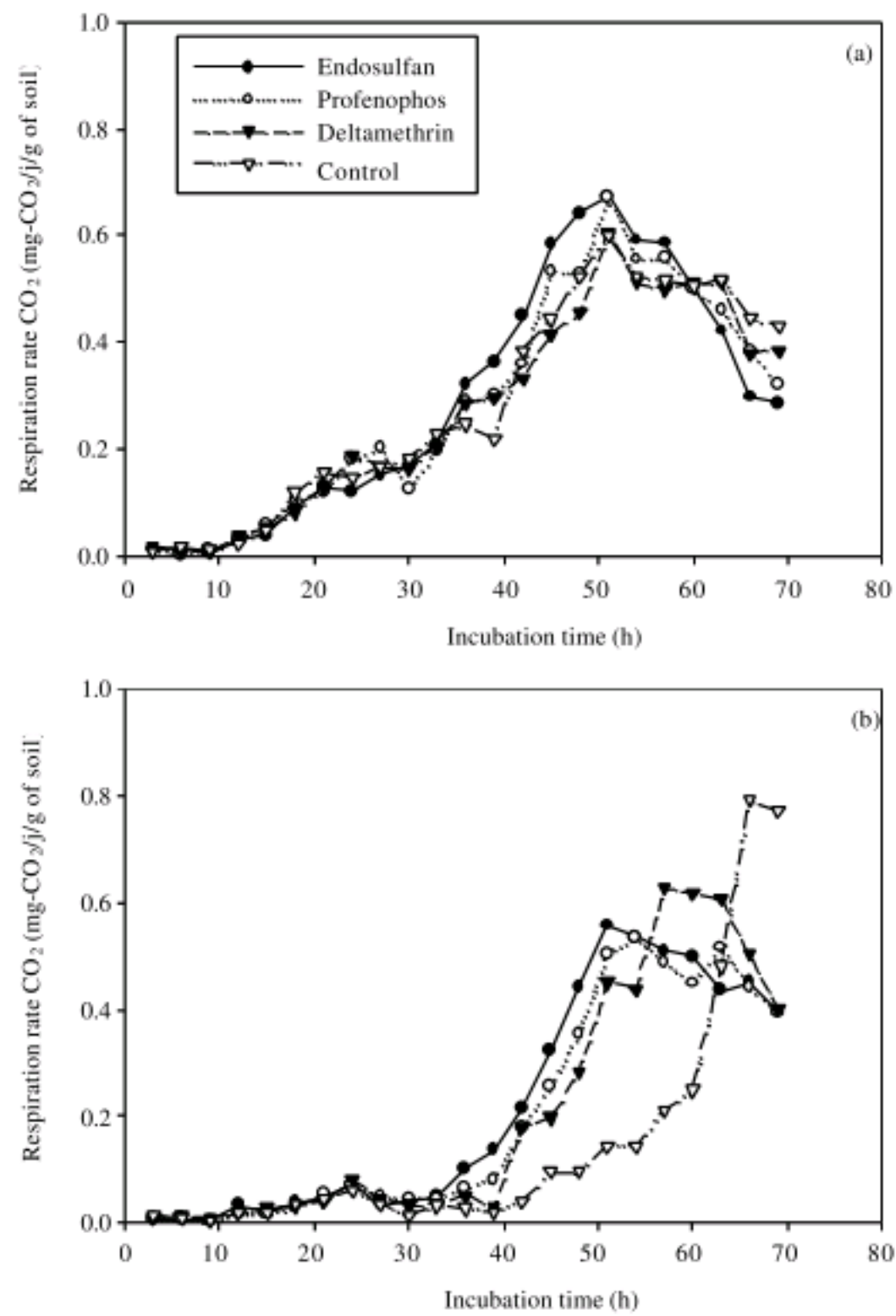


Fig. 3: (a) Microbial respiration kinetics of soils from cultivated plots and (b) fallow plot after addition of pesticides. Each point represents an average for the four field replicates. Note the difference in time scale with Fig. 2, here, CO<sub>2</sub> titrations were done every 3 h to show the short-term evolution

Endosulfan application decreased BR as indicated by the negative difference between BR before and BR after the application of the pesticide and referred to as BR-diff (Table 1, 2). On the other hand, a BR-diff>0 was observed with profenophos and deltamethrin applications and the control (Table 1). However, in the two soil, endosulfan application led to the highest reduction of BR (most negative value of BR-diff) while highest increase was observed with the control treatment.

The SIR observed with cultivated soil was 0.5 time lower than that of the fallow soil for all applied pesticides (Table 2). The SIR varied from 0.014 to 0.016 mg-CO<sub>2</sub> g<sup>-1</sup> soil and 0.026 to 0.034 mg-CO<sub>2</sub> g<sup>-1</sup> soil in cultivated soil and fallow soil, respectively. However, for the same soil, pesticide application did not affect significantly the SIR compared to the control.

Short lag time (11-14 h) was observed with cultivated soil as compared with soil from fallow plot (53-69 h) (Table 1, 2 and Fig. 3). Pesticide application did not affect significantly

Table 1: Effect of pesticides on respiration parameters of soil from cultivated plot

Pesticides	BR-diff	SIR	Lag time	Rmax	Cumulative CO <sub>2</sub> evolved 1	Cumulative CO <sub>2</sub> evolved 2	Q
Endosulfan	-0.009±0.005 <sup>a</sup>	0.016±0.0005 <sup>a</sup>	13.5±3.0 <sup>a</sup>	0.675±0.03 <sup>a</sup>	0.127±0.053 <sup>a</sup>	15.20±0.8 <sup>a</sup>	2.07±1.05 <sup>a</sup>
Profenophos	0.0110±0.02 <sup>ab</sup>	0.015±0.006 <sup>a</sup>	12.7±2.9 <sup>a</sup>	0.685±0.04 <sup>a</sup>	0.220±0.157 <sup>a</sup>	15.55±1.0 <sup>a</sup>	1.65±1.69 <sup>a</sup>
Deltamethrin	0.0060±0.02 <sup>ab</sup>	0.015±0.0045 <sup>a</sup>	12.0±2.4 <sup>a</sup>	0.670±0.095 <sup>a</sup>	0.350±0.177 <sup>a</sup>	15.20±0.6 <sup>a</sup>	2.77±0.69 <sup>a</sup>
Control	0.0275±0.014 <sup>b</sup>	0.014±0.0001 <sup>a</sup>	11.2±1.5 <sup>a</sup>	0.665±0.03 <sup>a</sup>	0.336±0.099 <sup>a</sup>	15.20±0.6 <sup>a</sup>	2.62±1.25 <sup>a</sup>

In a same column, values affected with the same letter are not significantly different at p<5%. Cumulative CO<sub>2</sub> evolved 1: Cumulative CO<sub>2</sub> evolved between pesticide application and nutrient addition (7 days). Cumulative CO<sub>2</sub> evolved 2: Cumulative CO<sub>2</sub> evolved from nutrients addition until the end of the experiment (25 days)

Table 2: Effect of pesticides on respiration parameters of soil from fallow plot

Pesticides	BR-diff	SIR	Lag time	Rmax	Cumulative CO <sub>2</sub> evolved 1	Cumulative CO <sub>2</sub> evolved 2	Q
Endosulfan	-0.0050±0.010 <sup>a</sup>	0.026±0.009 <sup>a</sup>	28.5±5.2 <sup>a</sup>	0.620±0.045 <sup>a</sup>	0.093±0.055 <sup>a</sup>	15.90±0.35 <sup>ab</sup>	0.95±0.77 <sup>a</sup>
Profenophos	-0.0020±0.005 <sup>a</sup>	0.028±0.095 <sup>a</sup>	29.2±2.9 <sup>a</sup>	0.645±0.07 <sup>ab</sup>	0.168±0.077 <sup>ab</sup>	16.35±0.35 <sup>b</sup>	0.85±0.55 <sup>a</sup>
Deltamethrin	0.0045±0.015 <sup>a</sup>	0.034±0.005 <sup>a</sup>	37.5±1.7 <sup>b</sup>	0.655±0.07 <sup>b</sup>	0.329±0.096 <sup>b</sup>	15.90±0.35 <sup>ab</sup>	1.18±0.60 <sup>a</sup>
Control	0.0160±0.014 <sup>a</sup>	0.027±0.001 <sup>a</sup>	42.0±4.2 <sup>b</sup>	0.830±0.11 <sup>c</sup>	0.216±0.110 <sup>ab</sup>	15.30±0.75 <sup>a</sup>	0.91±0.17 <sup>a</sup>

In a same column, values affected with the same letter are not significantly different at p<5%. Cumulative CO<sub>2</sub> evolved 1: Cumulative CO<sub>2</sub> evolved between pesticide application and nutrient addition (7 days). Cumulative CO<sub>2</sub> evolved 2: Cumulative CO<sub>2</sub> evolved from nutrients addition until the end of the experiment (25 days)

the lag time with cultivated soil, while with fallow soil, pesticide reduced significantly the lag time as compared with the control.

In general, with cultivated soil, pesticide did not affect the maximum respiration rate (Rmax). But with fallow soil, pesticides reduced Rmax. Indeed, Rmax were 0.032 mg-CO<sub>2</sub> g<sup>-1</sup> soil in presence of pesticide and 0.042 mg-CO<sub>2</sub> g<sup>-1</sup> soil in absence of pesticide (control). In addition, in the two soils, pesticide application anticipated the apparition Rmax compared to the control (Fig. 3).

Whatever the soil, cumulative CO<sub>2</sub> respired between the application of the pesticides and the one of the nutriments was higher with the profenophos and lowest with the control. The respiratory quotient (Q) was higher with cultivated soil compared to fallow soil.

## DISCUSSION

Results from this study allowed evaluating the impact of endosulfan, deltamethrin and profenophos on microorganism respiration parameters of soil from different land use. The effect of the pesticides on soil microbiological and biological properties was the main issues of research because of their impacts on public health and the quality of the environment. Basal respiration which is believed to reflect the potential of the microbial activity showed a tendency to be higher in the cultivated soil as compared to fallow. A possible explanation is that, although the application of compost in the cultivated soil led to OM content 1.5 times higher than that of the fallow soil, the soluble (available) carbon content that is the microbial energy source had not increase significantly. This was probably due to the use of too much mature compost by the farmers in the study site. Present results are in agreement with those reported by Perucci *et al.* (2000) and Chowdhury *et al.* (2008) indicating that organic amendments increase the microbial biomass and therefore the BR. Endosulfan inhibits microbial activity leading to a reduction of BR as observed with the soil with cultivated soil having high OM content (Ghadiri *et al.*, 2001; Iqbal *et al.*, 2001; Martin *et al.*, 2003). On the other hand, the slight increase of BR following profenophos and deltamethrin application has also been reported by Tejada *et al.* (2001) and would be probably due to the use of these pesticides as source of nutriments and energy by soil microorganisms (Iqbal *et al.*, 2001). On agricultural soils, Wardle and Parkinson (1990) showed an increase of BR following

application of herbicides (2, 4-D, picloram and glyphosate) at the rate of 200 mg kg<sup>-1</sup> of soil. In addition, application rate in this study would be very low to be toxic for soil microorganisms. Indeed, previous works reported that application of some pesticides doesn't depress soil microorganisms population and activity (Savadogo *et al.*, 2009), except the rate excessively superior to those applied in the field (Tu and Miles, 1976; Kale and Raghu, 1989). Although, we didn't study pesticide degradation, the slight reduction of the cumulative CO<sub>2</sub> observed between the application of the pesticides (endosulfan, profenophos) and the one of the nutrients indicated that these two pesticides tend to have negative effects on soil microorganisms metabolism. On the other hand, cumulative CO<sub>2</sub> was higher with the deltamethrin compared to the control, indicating that this pesticide had been used as nutrient. These findings were consistent with cumulative CO<sub>2</sub> calculated from nutrients application until the end of the experimentation that didn't show difference between the treatments with cultivated soil. However, with fallow soil it was higher with profenophos.

The short half-life of the pesticides used in this study also contributes to explain our results. More the half-life of the pesticide is short and more the pesticide doesn't have an effect on the microbial activity.

The Substrate Induced Respiration (SIR) measure the soil microbial biomass which is physiologically active (Anderson and Domsch, 1978).

Present results indicated a small proportion of the microorganisms physiologically active in the cultivated soil compared to fallow. An explication to that could be an inhibitory or toxic effect or a shift in microbial community due to the repeated application of the pesticides in the cultivated soil (Anderson *et al.*, 1981; Ghadiri, 2001). Indeed, repeated application of the pesticides lead to the selection and/or the adaptation of the soil microorganisms in response to applied pesticides or their homologues. Therefore, it would have developed active autochthonous microbial population able to degrade pesticides. Fallow soil had never received an application of pesticides, then, the first application of pesticides (in this study) certainly, would have cause important mortality of soil microorganisms. The dead microorganisms' cell would have served as nutrients for growth of resistant microorganisms, dragging higher active soil microbial biomass in fallow soil.

The lag time is believed to reflect modification or not the microbial population physiology showed that fallow soil was more affected by pesticides than cultivated soil. The fast response of the microorganisms to the addition of the nutrients (C, N and P) in the cultivated soil (short lag time of 11 h) was also reported by Schnürer (2006) with Oh horizon soil samples (in Sweden) and receiving pesticide application (2,6-dichlorobenzamide and 2,6-dichlorobenzonitrile) and then of nutrients (C, N and P). With parkland agroforestry soils in Burkina Faso, without application of pesticide, Gnankambary *et al.* (2007) noted the same range of lag time than that of the cultivated soil and has been attributed to the zymogenous microorganisms recognized to react very fast to the addition of energy rich compound.

The difference between lag times of the two soils could suggest a difference in microbial community induced by the land use. The reduction in lag time following pesticide application in soil without any experience of pesticides (fallow soil) supported this hypothesis. On the other hand, findings from previous studies showed that lag time increases with the pesticide toxicity or its residues quantity (Martin *et al.*, 2003; Schnürer, 2006).

The respiratory quotient (Q) reflects the level of soil pollution (Anderson and Domsch, 1985; Insam and Domsch, 1988). The higher Q confirmed that cultivated soil had high pollution degree than fallow soil. Repeated use of these pesticides with high half-life in cultivated soil led to an accumulation of high amount of pesticide residues.



In conclusion, this study has shown that repeated application of pesticides in the field affected soil microorganisms' respiration parameters. Endosulfan decreased basal respiration while profenophos and deltamethrin increased it. The lag time was shorter with cultivated soil, indicating clearly soil with cultural history with pesticide application. Basal respiration and lag time could be useful tools to discriminate soil quality in semi-arid condition and to assess the impact of pesticides on soil biology.

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