



EFFECT OF FIPRONIL, A PYRAZOLE INSECTICIDE, ON MICROBIAL BIOMASS CARBON, SOIL RESPIRATION, FDA AND DEHYDROGENASE ACTIVITY OF SOIL

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ABSTRACT

A laboratory incubation experiment was conducted with fipronil, a pyrazole insecticide, to study its effect on microbial biomass carbon, soil respiration, fluorescein diacetate hydrolyzing activity (FDA) and dehydrogenase activity in the alluvial soil. Fipronil was incorporated into soil sample followed by incubation and sampling for different periods of time (1, 3, 7, 15, 30, 45 and 60 days) at FR (field application rate) (0.675 μgml^{-1}), 2FR (1.35 μgml^{-1}) and 10FR (6.75 $\mu\text{g ml}^{-1}$) along with one set of control at 25°C-30°C under 60% water holding capacity. Application of fipronil showed no inhibitory effect on microbial biomass carbon, soil respiration, FDA and dehydrogenase activity when applied at recommended dose in alluvial soil.

KEYWORDS: Fipronil, MBC, Soil Respiration, FDA, dehydrogenase activity, alluvial soil.

INTRODUCTION

The wide spread use of pesticides is very common in agricultural crop production regarding yield and quality of crops (Crum *et al.*, 1999; McDonald *et al.*, 1999). Increasing pesticide usage over time raises a concern for the environmental contamination and causes negative effects on human health (McDonald *et al.*, 1999; Perucci *et al.*, 2000, Zhu *et al.*, 2004). The effects of pesticide usage must be monitored in the context of soil pollution and sustainability of the agro-ecosystem. However, as crop protection chemical, the use of synthetic pesticide cannot be fully substituted in modern agriculture for assured crop production. The transformation of pesticides by soil micro-organisms consists in essentially intracellular enzyme-catalyzed reactions encountered in their regular metabolic activity (Wu and Nofziger, 1999). Fipronil, the first phenylpyrazole introduced for pest control (Hainzl and Casida, 1996). Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4(trifluoromethyl) sulfinyl] -1H-pyrazole was discovered by Rhone-Poulenc Agro (currently Aventis Crop Sciences) in 1987, introduced in 1993, and registered as a pesticide in the U.S in 1996 (Bobeet *al.* 1998a). The mode of action involves blocking the γ -amino butyric acid (GABA)-gated chloride channel, thereby disrupting central nervous system activity and, at sufficient doses, causing death (Cole *et al.*, 1993). Safe use of an agrochemical depends on its toxicological properties and its distribution and persistence in the environment. Impact of application of an insecticide can be monitored by assessment of soil microbiological parameter and enzymatic activity in soil.

Microbial biomass Carbon (MBC) contribute a variable but significant portion to the active pools of soil C and N; determination of MBC provides better insights of soil organic C turnover (Wang *et al.*, 2004). Microbial biomass

and activities can be influenced by several ecological factors, such as plant community composition, soil organic matter level, soil moisture, and temperature (Li and Chen, 2004). Understanding the environmental influences on microbial biomass and activity is the key to predicting changes in nutrient cycling (Arnold *et al.*, 1999). Determination of soil respiration is the appropriate method to determine the general metabolic activity of soil microorganisms (Nannipieriet *al.*, 1990). Respiration of unamended soil is termed as basal respiration, which gives a potential measure of quantitative microbial activity (Gray, 1990) and is considered as bio-indicators of soil quality (Gregorich *et al.*, 1994).

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Burns 1983; Sinsabaugh *et al.*, 1991). They are important in catalysing several important reactions necessary for the life processes of micro-organisms in soils and the stabilisation of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick *et al.*, 1994). These enzymes are constantly being synthesized, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling (Tabatabai, 1994; Dick, 1997). A better understanding of the role of these soil enzymatic activities in the ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices (Dick, 1994; Dick, 1997; Bandick and Dick, 1999).

Fluorescein diacetate hydrolyzing activity (FDA) is simple, sensitive and rapid method to determine the total microbial activity in soil. FDA is hydrolyzed by a number

of different enzymes, such as proteases, lipases and esterases, produced by soil microflora. The product of this enzymatic conversion is fluorescein.

Soil dehydrogenase activity is considered to be a valuable parameter for assessing the side effects of pesticide treatments on the soil microbial biomass. Studies on the activity of dehydrogenase enzyme in the soil is very important as it may indicate the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

The purpose of the present study was to provide information about the effect of, fipronil on microbial biomass carbon, FDA, dehydrogenase activity and respiration of soil under different treatment doses.

MATERIALS & METHODS

Collection of soil sample

Field-moist alluvial soil sample (pH 8.3 and moisture content 19.5%) was collected from 0-15 cm depth from Kalyani, Nadia, West Bengal. The soil was clay-loam in texture. The physico-chemical properties of the soil were- 0.92% organic C, pH 7.9, EC 0.0017 (dsm^{-1}) and water holding capacity 75.7%. The soil had no history of receiving any pesticide treatment six months prior to this study. The soil samples were properly labeled and sealed with polythene bags.

Preparation of the soil sample

After collection of the sample large lumps of soil were broken, root exudates were separated and spread out in a large plastic tray to become air dried to perform the study. Then it was grinded in a mortar under such conditions that the aggregate particles were crushed but no actual grinding of the ultimate particles of the soil occurs. Sufficient water was added to bring the soil samples to 60% of maximum water holding capacity and stabilized at 25 °C in the dark for one week

Treatment of fipronil in soil

A 5% EC formulation of fipronil obtained from Agro Pvt. Ltd. Three stock solutions containing 0.675 $\mu\text{g/ml}$ (FR), 1.350 $\mu\text{g/ml}$ (2FR), and 6.75 $\mu\text{g/ml}$ (10FR) of pyrazole insecticide were prepared in distilled water. We now added the various rates to the 100 g of accurately weighed soil and mixed thoroughly with glass rod and set for incubation. Three treatment doses of fipronil, 0.675 μgml^{-1} as field rate (FR), 2-fold the field rate *i.e.* 1.35 μgml^{-1} (2FR) and 10-fold the field rate *i.e.* 6.75 μgml^{-1} (10FR) were applied in three replicated soil samples with running a set of control where no dose of insecticide was applied. The 10FR is recommended in laboratory tests to assess the side effect of insecticide on soil microflora (Sommerville, 1987). To study the influence of the insecticide the periodic changes of microbial biomass carbon (MBC), soil respiration, FDA and dehydrogenase activity in soil had been observed with increasing treatment doses of fipronil at 1, 3, 7, 15, 30, 45 and 60 days of incubation.

Study of soil microbial biomass carbon (MBC)

Microbial biomass carbon was determined by fumigation extraction method (Joergensen, 1995) followed by determination of K_2SO_4 extractable C (Vance *et al.*, 1987)

by using the relationship, $\text{MBC} = 2.64 \text{ Ec}$, where Ec was obtained by subtracting the value of the K_2SO_4 -extractable C of the unfumigated soil from that of the fumigated soil.

Study of soil respiration

To estimate soil respiration three portion of 10 g moist soil was accurately weighed and taken in three incubation flasks. 1 ml of water was added to the soil. 5 ml 0.1 (M) NaOH was pipetted out into a vial and the same was inserted into the flask and closed by air tight cork. The samples as well as the blank flasks were incubated at 22°C for 24 hours. After the expiry of incubation, left over NaOH solution was quantitatively transferred to a 50 ml conical flask with 10 ml distilled water and titrated with 0.05 (M) HCL in presence of 5 ml BaCl_2 solution and three drops of phenolphthalein indicator solution.

Study of FDA in soil

The estimation of FDA was based on Incubation of soil with sodium phosphate buffer (60 mM, pH 7.6) and 1 ml of fluorescein diacetate solution (240 $\mu\text{g/ml}$) for three hours at 24°C followed by taking absorbance at $\lambda = 490 \text{ nm}$ with UV-VIS spectrophotometer (ELICO Model No. SL 171) as reported by Alef (1995b).

Study of dehydrogenase activity in soil

Determination of soil dehydrogenase activity in soils is based on the use of soluble tetrazolium salts [2,3,5-triphenylotetrazolium chloride (TTC)], as artificial electron acceptors, which are reduced to red-coloured formazans, extracted and then determined calorimetrically (Casida *et al.*, 1964). 20g of moist soil and 0.2g CaCO_3 was mixed thoroughly followed by 5ml double distilled water and 1 ml of 3% TTC are mixed and incubated at 37°C for 24 hours. The mixture was then extracted with methanol and absorbance was measured at $\lambda = 185 \text{ nm}$ with ELICO-SL-171 UV-VIS spectrophotometer.

RESULTS & DISCUSSION

An increase in soil MBC was observed at FR (572.9 $\mu\text{g g}^{-1}$) in compared to 2FR (511.7 $\mu\text{g g}^{-1}$) and 10FR (445.4 $\mu\text{g g}^{-1}$) application rates (Table 1) when fipronil applied in soil. It indicated that treatments higher than recommended application rate had detrimental or toxic effect on MBC of incubated soil. It can be stated that the excessive application of pesticides decreases the number of microorganisms in soil. Considering the incubation period, there was a gradual increase in soil MBC upto 15 days of incubation after fipronil application indicating the positive effect of insecticide (Fig 1). This increase in soil microbial biomass might be due to the fact that the pesticide or the dead microbial cells killed by it, served as a carbon or, energy source by the surviving micro-organisms (Handa *et al.*, 1999) required for cell proliferation. But an inhibitory effect of application of fipronil was observed after 30 days of incubation (Fig 1) though it minimized later after 60 days. Reports on the effects of pesticides (2, 4-D, Imazethapyr, alachlor *etc.*) on soil microorganism were in agreement with our results (Rath *et al.*, 1998, Perucci and Scarponi, 1994, Pozo *et al.*, 1994) where pesticides did not show any adverse effects on soil microbial biomass.

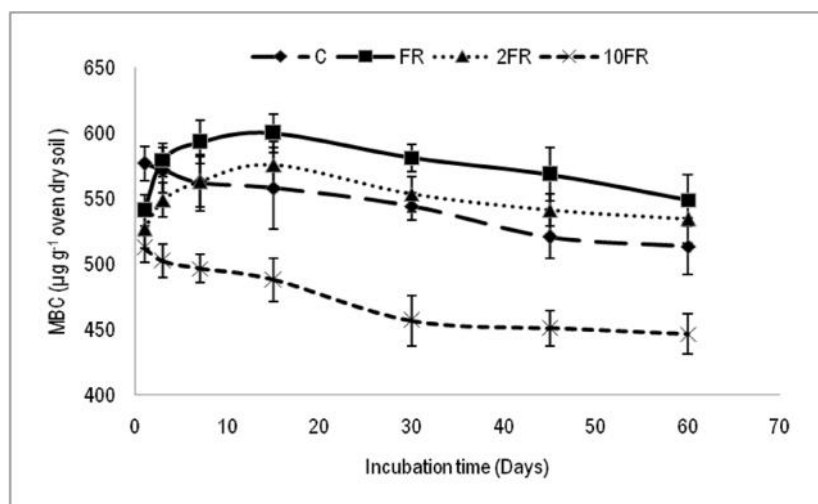


FIGURE 1. Variation of MBC with treatment and incubation days

TABLE 1: Variation of MBC, soil respiration, FDA and dehydrogenase activity with treatment and incubation days after application of fipronil

Treatment	Incubation Time (Days)						Mean	SD	
	1	3	7	15	30	45			60
MBC ($\mu\text{g g}^{-1}$ oven dry soil)									
C	576.6	571.6	562.1	557.8	544.0	520.5	513.4	549.4	24.6
FR	540.5	579.3	592.9	599.7	581.0	568.4	548.7	572.9	21.9
2FR	526.8	548.6	562.8	575.5	553.3	541.0	534.2	548.9	16.8
10FR	511.9	502.1	496.3	487.6	456.5	450.7	446.4	478.8	27.0
Soil respiration ($\mu\text{g g}^{-1}$ oven dry soil hr^{-1})									
C	2.09	2.07	1.95	1.77	1.66	1.43	1.30	1.75	0.31
FR	1.86	1.97	2.03	2.12	1.92	1.65	1.59	1.88	0.19
2FR	1.79	1.92	1.93	1.90	1.74	1.56	1.47	1.76	0.18
10FR	1.68	1.59	1.46	1.37	1.13	1.18	1.20	1.37	0.21
FDA ($\mu\text{g g}^{-1}$ oven dry soil hr^{-1})									
C	70.7	69.6	68.8	67.4	60.4	59.6	57.7	64.9	70.7
FR	69.3	72.8	76.7	77.9	72.6	69.6	63.3	71.7	69.3
2FR	64.4	67.8	69.7	73.6	67.7	65.7	60.7	67.1	64.4
10FR	64.9	60.8	57.5	55.3	49.7	50.3	49.9	55.5	64.9
Dehydrogenase activity ($\mu\text{g g}^{-1}$ oven dry soil hr^{-1})									
C	62.9	61.9	59.7	59.2	54.6	53.3	50.4	57.4	4.70
FR	57.3	62.4	67.8	68.6	63.4	61.8	58.5	62.8	4.27
2FR	54.8	59.1	62.7	64.3	59.8	55.3	53.7	58.5	4.09
10FR	53.6	52.8	50.7	49.8	44.9	46.7	47.2	49.4	3.26

With application of fipronil, soil respiration rate found to increase in case of FR ($1.88\mu\text{g g}^{-1}$) and 2FR ($1.76\mu\text{g g}^{-1}$) over control ($1.75\mu\text{g g}^{-1}$) whereas decreased for 10FR ($1.37\mu\text{g g}^{-1}$) suggesting inhibitory effect of insecticide. (Table 1). During incubation of soil sample, greater CO_2 was released from soil with added insecticide over the control soil. The initial respiration rate increased on the first day and rate of soil respiration maintained higher values upto 15 days. Then, a decrease in CO_2 was evidenced upto 60 days (Fig 2). This phenomenon can be further supported by strong positive correlation value ($r=0.783^{**}$) between MBC and soil respiration. Das et al. (2007) reported the same trend when the effect of novaluron in soil respiration was studied.

Overall microbial activity in soil can be assessed by determining fluorescein diacetate hydrolyzing activity (Nannipieri *et al.*, 1990 and Dick, 1994). FDA might be considered as a suitable tool for measuring the early detrimental effect of pesticides on soil microbial biomass (Perucci *et al.*, 2000). It was seen that FDA decreased with

progression of days of incubation and with increase of treatment doses over control. FDA activity was increased upto 15 days of incubation and then decreased after that period with time (Fig 3). It indicated that FDA in soil increased when fipronil applied at FR ($71.7\mu\text{g g}^{-1}$) and even at 2FR ($67.1\mu\text{g g}^{-1}$) over control ($64.9\mu\text{g g}^{-1}$) while it showed significant detrimental effect for 10FR ($55.5\mu\text{g g}^{-1}$) applied to the soil under study (Table 1). Active microbial cells transport fluorescein diacetate inside the cell where it is hydrolyzed into polar fluorescein, which is released in the extracellular environment (Alef, 1995b). It was observed that FDA followed a similar trend like microbial biomass carbon and soil respiration which could be supported by the strong positive correlation among three parameters. It showed a positive correlation finding $r=0.802^{**}$ between MBC and FDA and $r=0.781^{**}$ between soil respiration and FDA. Similar result was found when Imazethapyr applied at field rate, under field and laboratory studies (Haney *et al.*, 2002).

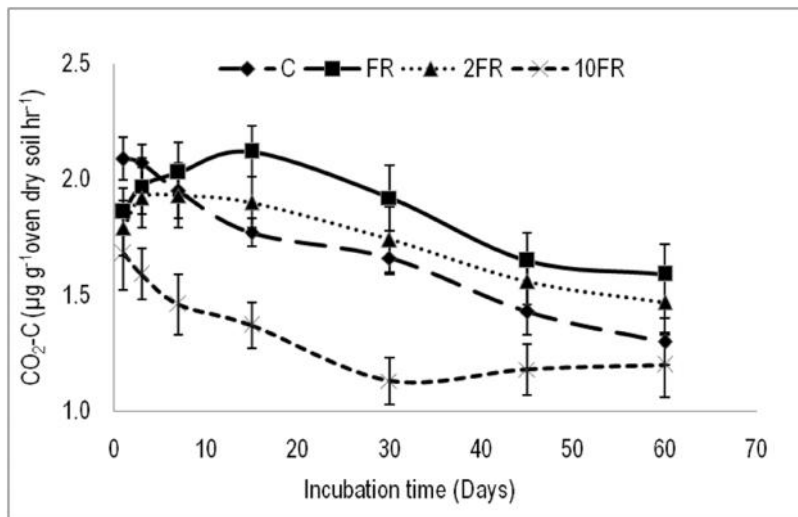


FIGURE 2. Variation of soil respiration with treatment and incubation days

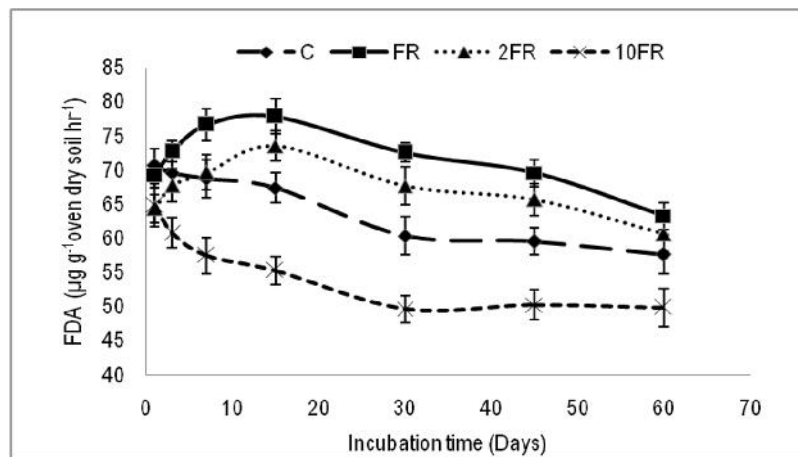


FIGURE 3. Variation of FDA with treatment and incubation days

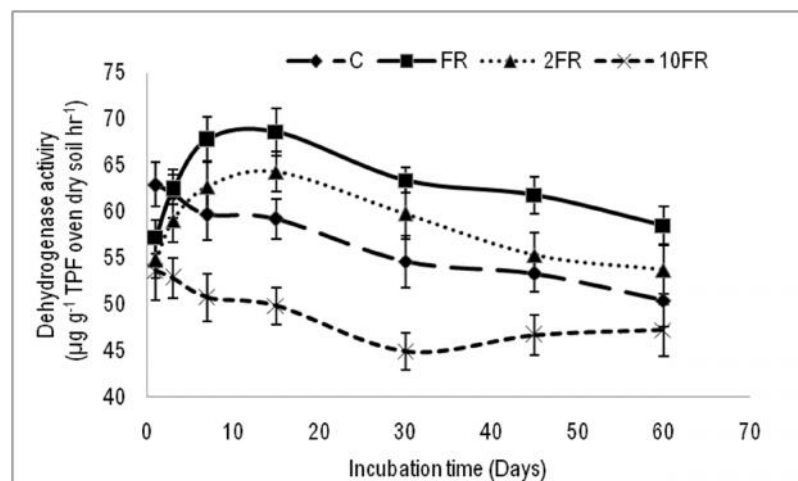


FIGURE 4. Variation of dehydrogenase activity with treatment and incubation days

Dehydrogenase activity followed the similar trend with soil fluoroscene hydrolysing activity with application of fipronil. It was observed to increase for FR (62.8.7µg g⁻¹) and 2FR (58.5µg g⁻¹) application of insecticide in compared with the control one (57.4 µg g⁻¹) (Table 1) while it showed inhibitory effect 10FR (49.4µg g⁻¹). For

soil dehydrogenase activity, this trend was followed upto 15 days of incubation and then observed to increase slowly (Fig 4). On the first day of the laboratory experiment low values of dehydrogenase activity were found whereas the final days of incubation resulted in higher value of dehydrogenase activity at all treatment doses of fipronil

indicating that effect of the insecticide was minimized in progress of incubation time. Similar result was found when the effect of fonofos on dehydrogenase activity was evaluated taking into consideration the pesticide dose and soil incubation time (Stępniewska et al., 2006). It showed a positive correlation having $r = 0.769^{**}$ between MBC and dehydrogenase, $r = 0.912^{**}$ between soil respiration and dehydrogenase, $r = 0.802^{**}$ between FDA and dehydrogenase activity.

CONCLUSION

The result of the study indicated that application of pyrazole insecticide fipronil in soil did not show any significant inhibitory effect at recommended field dose or double of the recommended field dose, but it imparted detrimental effect at 10FR dose on MBC, soil respiration, FDA and dehydrogenase activity upto 15 days of incubation whereas the effect was minimized with time. From the present study it can be concluded that the incorporation of fipronil at recommended doses to the alluvial soil has no long term harmful effects on soil microbial activity and in turn in soil.

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