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# Response of Soil Microflora, Microbial Biomass and Some Soil Enzymes to Baythroid (An Insecticide)

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**Abstract:** Laboratory incubation experiments were conducted to study the response of bacterial and fungal population, soil microbial biomass, urease, amylase, invertase and cellulase to Baythroid applied at 0, 0.4, 0.8, 1.6, 3.2 and 6.4 pg $g^{-1}$ soil (on an active ingredient basis). Generally, a positive effect on bacterial and fungal population was observed. 'Bacterial population increased from 13 to an average of 25 after 5 days of incubation of soil samples treated with different levels of Baythroid. Baythroid did not have a significant effect on fungal population, which was quite low after 5 days of incubation. After 15 days of incubation, however, Baythroid caused a substantial increase in fungal population although no consistent trends were observed with the rate of application.

Carbon dioxide evolution from soil was almost unaffected by Baythroid except at the lowest and the highest levels of addition, where a negative and a positive effect, respectively, was obvious. Cumulative losses of $CO_2$-C increased by 38% at the highest level of Baythroid. The microbial biomass C varied between 138 and 147 pg $CO_2$-C $g^{-1}$ soil in differently treated soils, a substantially positive effect of Baythroid was observed only at the highest rate of addition, while at lower levels a positive but non-significant effect was observed. Amylase activity increased by a maximum of 91.5% at Baythroid level of 1.6 $\mu$g $g^{-1}$. At 6.4 $\mu$g $g^{-1}$ soil Baythroid, however, the activity was reduced by 47.9%. Invertase activity also increased by 110.9% at 1.6 $\mu$g Baythroid $g^{-1}$ soil followed by a decrease of 40.3% at the highest level tested. Cellulase activity was not much affected, although an increase of 18.5% was observed at 1.6 pg $g^{-1}$ soil Baythroid. At the highest level of Baythroid, however, cellulase activity was reduced by 25.9%. Response of urease was almost similar to that of other enzymes. However, maximum increase of 40.9% was achieved at 0.8 pg $g^{-1}$ soil Baythroid, while the decrease (9.1%) at higher levels of Baythroid was less pronounced as compared to that for other enzymes. All the four enzymes showed a positive relationship in their response to different rates of Baythroid.

**Keywords:** Amylase, Baythroid, Cellulase, Insecticide, Invertase, Microbial biomass, Urease

## Introduction

There has been considerable concern about the effects of pesticides on the soil microflora since the beginning of pesticide use (Greaves and Malkomes, 1980). This is because of the observation that a part of the material always reaches the soil during spray application or along with rain or dew water, which may influence soil microflora and their functions leading to changes in the nutrient availability to plants (El-Abyad and Ghareeb, 1991). However, the effect of pesticides on soil microflora may be only slight and short lived at optimum rates of application (Schuster and Schroder, 1990).

2Among different microbial activities in soil, decomposition of organic matter, microbial growth, ammonification, nitrification, denitrification and $N_2$ fixation are of particular interest due to their influence on nitrogen availability to plants. Both positive and negative effects on microbial composition and activity have been reported (Schuster and Schroder, 1990; Katayama and Kuwatsuka, 1991; Martinez-Toledo *et al*., 1992a,b).

In Pakistan, a major chunk of pesticides consists of insecticides, which are mostly used on cotton. Of the different kinds of insecticides, synthetic pyrethroids have become a commercially successful group; Baythroid (a product of Bayer, Germany) being an example of such insecticides. Cyfluthrin ($C_{22}H_{18}Cl_2FNO_3$) is the common name of the active ingredient of Baythroid. Previously, we have reported on the influence of Baythroid on ammonification-nitrification of organic and inorganic N, mineralization-immobilization turnover of inorganic N (Lodhi *et al*., 1994) and on nitrification inhibition (Lodhi *et al*., 1996c). Studies have also been reported on the effect of baythroid on plant growth and uptake of nutrients (Lodhi *at al*., 1996a,b). This paper describes some results on the effect of Baythroid on bacterial and fungal population, soil microbial biomass, urease, amylase, invertase and cellulase.

## Materials and Methods

The soil was collected from experimental fields at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Air-dried and sieved soil had pH, 7.8; sand, 19%; silt, 40%; clay, 41%; WHC (water-holding capacity), 32.2%; organic C, 0.44%; total N, 0.05%; mineral N ($NH_4 + NO_3 + NO_2$), 18.5 $\mu$g $g^{-1}$ soil.

Baythroid is the trade name of an insecticide manufactured by Bayer, Germany. It is available in several formulations ranging from water soluble and emulsifiable concentrates to granular forms. In Pakistan, water-soluble formulation of Baythroid is generally used for insects/pests of cotton. Commercial formulation of Baythroid obtained from the local market contained cyfluthrin and methamedophos at 25 and 500 g $L^{-1}$.

Air-dried and sieved (<2 mm) soil (50 g) was taken in 100 ml capacity plastic containers and treated with aqueous solution of Baythroid at 0, 0.4, 0.8, 1.6, 3.2 and 6.4 mg $g^{-1}$ soil (on an active ingredient basis). The treated samples were incubated at 30°C for 7 days and analyzed for bacterial and fungal population by dilution plate method (Booths, 1971). For fungal population, malt extract agar medium containing rose bengal (0.003%) was adjusted to pH 5.5, while for bacterial population, the medium without rose bengal was used after adjusting to pH 7.5. Triplicate plates were used for each determination. Bacterial counts were taken after 2 days and fungal counts after 5 days of incubation. Soil samples (50 g) contained in plastic containers were treated with 0, 0.4, 0.8, 1.6, 3.2 and 6.4 pg Baythroid $g^{-1}$ soil (6 replicates for each treatment) and incubated at 30°C for 20 days. Chloroform-fumigation-incubation method (Jenkinson and Powlson, 1976) was used for determinating of microbial biomass. Replicate samples of test soils were exposed to $CHCl_3$ vapours for 24 hrs. in a desiccator. Fumigated soil samples were subjected to repeated evacuations to remove $CHCl_3$. Sub-samples (10 g) from

both fumigated and untreated soils were transferred to serum bottles (125 ml); fumigated samples were inoculated with 0.1 g of untreated soil, and all the samples incubated for 10 days at 30°C with rubber stoppers on the tightly clamped bottles. Air samples were taken from the bottles after 5 and 10 days of incubation and analyzed for their $CO_2$-C content using Gasukuro Kogyo 370 gas chromatograph equipped with a thermal conductivity detector (t.c.d). Carbon dioxide was separated on a 2 m long column (i.d., 3 mm) filled with porapak Q using $N_2$ as carrier gas at a flow rate of 30 ml/min. The temperature of column, injector, and detector was 75, 110, and 75°C, respectively and the detector current was 100 mA. A 10 cm pre-column filled with $Mg(ClO_4)_2$ was used to avoid interference of $H_2O$ vapour. The detector was calibrated with $CO_2$ standard (Gasukuro, Kogyo, Japan). Microbial biomass C was calculated as:

$$B = F/k$$

where, B is the microbial biomass C content, F is the flush of decomposition ($CO_2$-C evolved from fumigated minus that from untreated soil) and k is a constant that denotes proportion of microbial biomass mineralized to $CO_2$. In fumigated soils, $CO_2$-C values were those evolved in the 0-10 days incubation period whereas in unfumigated samples the chosen period was 10-20 days so as to avoid problems arising from soil disturbance. In the present study, k value of 0.45 was used (Oades and Jenkinson, 1979).

Soil samples (100 g) treated with Cyfluthrin at 0, 0.4, 0.8, 1.6, 3.2 or 6.4 pg $g^{-1}$, adjusted to 60% WHC moisture, incubated for 7 days at 30°C and assayed for urease, amylase, invertase, and cellulase activities. Urease activity was determined as described by Pancholy and Rice (1973) was followed for the assay of urease activity. Briefly, 1 ml toluene was thoroughly mixed with 10-g moist soil sample in a 100 ml Erlenmeyer flask. After 15 min, 20 ml of phosphate buffer (pH, 6.7) and 20 ml of 10% urea solution was added to the flask. The reactants were incubated at 30°C for 24-h followed by shaking for 15-min with 30 ml of 1 N KCl solution. The contents were filtered and the filtrate made upto 100 ml with deionized water. Aliquots (5 ml) were analyzed for the $NH_4$-N content. Urease activity was expressed as mg $NH_4$- N $g^{-1}$ soil.

Cellulase activity was determined as described by Pancholy and Rice (1973). Toluene-treated soil samples (as described for urease) were mixed with 20 ml 0.5 M acetate buffer (pH, 5.9) and 20 ml of freshly prepared 2% carboxymethyl cellulose (CMC). The soil mixture was incubated at 30°C for 24 h followed by centrifugation at 4000 rpm for 20-min. The supernatant was filtered through a Whatman No. 41 filtre paper and aliquots analyzed for the reducing sugars content using the DNS method of Gascoigne and Gascoigne (1958). Cellulase activity was expressed as mg reducing sugars produced soil 24 $h^{-1}$.

The method described by Ross (1966) for cellulase was slightly modified to determine amylase activity; a different pH (5.5) and 2% soluble starch were used in the assay. Amylase activity was expressed as mg reducing sugars produced $g^{-1}$ soil 24-$h^{-1}$. Same method was used to determine invertase activity except that the substrate was 20 ml of 5% sucrose solution. The enzyme activity was expressed as mg reducing sugars produced $g^{-1}$ soil 24-$h^{-1}$.

## Results and Discussion

Baythroid application had, in general, a positive effect on bacterial and fungal population. Bacterial population increased from $13x10^8$ to an average of $25x10^6$ after 5 days of incubation of soil samples treated with different levels of Baythroid. There was a considerable increase in bacterial population after 10 days followed by a decline after 15 days of incubation. However, positive effect of Baythroid was maintained throughout the incubation period. Baythroid did not have a significant effect on fungal population which was quite low after 5 days of incubation. A substantial increase in fungal population was observed after 10 days (an average of $2x10^4$ for different treatments after 5 days to an average of $21x10^4$ after 10 days) followed by a reduction during subsequent five days of incubation (average for different treatments being $19x10^4$). After 15 days of incubation, Baythroid did show a positive effect on fungal population but no consistent trends were observed with the rate of application (an average of $20x10^4$ for different Baythroid treatments compared to $14x10^4$ in control).

Different studies have shown wide variation in the response of soil microflora to pesticide application, both positive and negative effects being reported. Wainwright and Pugh (1973) reported an increase in the bacterial and fungal population of soil treated with fungicides. Gonzalez-Lopez *et al.* (1992) observed a significant increase in plate counts of both bacteria and fungi, the degree of stimulation was related to the concentration of acaricide. Martinez-Toledo *et al.* (1992a,b) found a decrease in the number of bacteria due to 10-300 $\mu$g $g^{-1}$ soil of Chlorpyrifos (an insecticide) but the fungal population was unaffected.

The variable response of soil microflora to xenobiotics may result from the differences in chemical nature and active concentration of the chemical used by different workers. In many cases, however, studies on the effect of pesticides on soil microflora have been interpreted using axenic cultures (Salmeron *et al.*, 1991), or have been performed by measuring microbial activities (Yeomans and Bremner, 1985). These effects may not be applicable to actual soil conditions. According to Greaves (1977), the effects observed are minor and short-lived so far as the total microbial population is concerned. Schuster and Schroder (1990) also reported that successive applications of the pesticides caused only slight and short-lived side effects on the soil microflora. The reasons for the effects being short-lived may be a gradual adsorption of the pesticide by the soil colloids making it ineffective so far as the microbial population is concerned. Generally, the pesticides are rapidly adsorbed on the organic and inorganic soil colloids. Gradual degradation of the pesticide may also be responsible for this effect. However, pesticides with lower rate of degradation may accumulate in soil leading to negative effects on soil microflora (Martinez-Toledo *et al.*, 1992a,b). in the present studies, cyfluthrin was found to be easily degradable and adsorbed fairly rapidly onto the soil colloidal complex rendering it non-extractable almost immediately after application (unpublished data). These observations could justify small and short-lived effects of Baythroid on microbial population. Data shows that loss of $CO_2$-C from untreated soil samples was almost unaffected by Baythroid except at the lowest and the highest levels of addition, where a negative and a positive effect, respectively, was obvious. This effect was observed at both the sampling intervals (10 and 20 days) although more losses of C occurred during the first 10 days as compared to the second 10 days. Cumulative losses of $CO_2$-C increased by 38% at the highest level of Baythroid. While a negative effect was not expected as observed at the lowest level of Baythroid probably arising from some error in treatment), a positive effect could be attributed to increased microbial activity, an observation in line with the results noted for N mineralization. Both positive and negative effects of pesticides on soil respiration have been reported. Harden *et al.* (1993) reported a negative effect of five

pesticides (benomyl, isoproturon, simazine, dinoterb, and chloroform) on microbial biomass. However, except for benomyl, other pesticides enhanced soil respiration during the 0-10 days incubation, the effects were less consistent during the 10-20 days period suggesting a loss of pesticidal effect. It also is likely that pesticides alter the population structure of microbial community in soil.

In the present study, loss of $CO_2$-C from fumigated samples was generally 5 times greater than that from untreated samples (average of different treatments being 80.2 and 14.2 $\mu$g $CO_2$-C $g^{-1}$ soil, respectively) leading to a flush of decomposition of 62-66 $\mu$g $CO_2$-C $g^{-1}$ soil. The microbial biomass C varied between 124 and 147 $\mu$g $CO_2$-C $g^{-1}$ soil in differently treated soils. A substantially positive effect of Baythroid on microbial biomass was observed only at 3.2 pg Baythroid $g^{-1}$ soil while at lower levels, a positive but non-significant effect was observed. Apparently, Baythroid caused an increase in the availability of C, which could not be attributed to Baythroid as a source of C because of fairly low levels of addition. Ghani *et al*. (1996) found that microbial biomass was unaffected by pesticide residues although respiration from untreated soils increased. This shows that pesticides may reduce the efficiency of soil microflora, by inducing the microbial biomass to waste C as $CO_2$ rather than using it for synthetic activities (Insam and Domsch, 1988). Katayama and Kuwatsuka (1991) observed a slight decrease in cellulose degradation as evidenced by low respiration. Turco and Konopka (1990) found a reduction in microbial biomass in soil treated with 100 pg $g^{-1}$ but not with 10 $\mu$g carbofuran $g^{-1}$ soil. Apparently, the side effects of normal rates of pesticide application on microbial biomass may be of little ecological significance as also suggested by Schuster and Schroder (1990). Pesticide molecules may accumulate in specific microhabitats, but it is highly unlikely that concentrations ever reach effective levels after a single application of pesticide.

Data shows the response of different enzyme activities to the Baythroid application. Amylase activity increased by a maximum of 91.5% at Baythroid level of 1.6 $\mu$g $g^{-1}$. At 6.4 pg soil Baythroid, however, the activity was reduced by 47.9%. Invertase activity also increased by 110.9% at 1.6 $\mu$g Baythroid $g^{-1}$ soil followed by a decrease of 40.3% at the highest level tested. Cellulase activity was not much affected, although an increase of 18.5% was observed at 1.6 $\mu$g.

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