Pak J Sci Ind Res 1998 41 (4) 212-216

INHIBITION OF NITRIFICATION IN SOIL BY BAYTHROID AND ITS COMPARISON WITH N-SERVE

Asma Lodhi[a], *N N Malik*[b] *and F Azam*[a*]

[a]*Soil Biology Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*
[b]*Institute of Chemistry, University of Punjab, Lahore, Pakistan*

(Received 19 August 1996; accepted 18 February 1998)

In laboratory experiments, Baythroid (an insecticide produced by Bayer, West Germany) was compared with N-Serve (a well-known nitrification inhibitor) for its effect on the process of nitrification in soil. In one experiment, soil samples were incubated for 7 weeks at 30°C after treatment with Baythroid (0.4, 1.6, or 6.4 μg g^{-1} soil) or with N-Serve (1.1, 4.4, and 17.6 μg g^{-1} soil); 200 μg g^{-1} soil N as ammonium sulphate was applied in all cases. Soil samples analyzed at different incubation intervals revealed significant inhibition of nitrification and accumulation of NH_4-N due to both the chemicals and the effects increased with the rate of application. At lower levels of inhibitors, the effect was short-lived, while at higher levels limited amounts of NO_3-N were observed after 7 weeks of incubation. N-Serve was found to be more inhibitory to nitrification as compared with Baythroid. In another experiment where soil samples treated with Baythroid (at 0.4, 0.8, 1.6, 3.2, or 6.4 μg g^{-1} soil) and N-Serve (1.1, 2.2, 4.4, 8.8, or 17.6 μg g^{-1} soil) were incubated for 10, 20 and 30 days prior to addition of N (200 μg g^{-1} soil as ammonium sulphate), N-Serve was found to be more persistent in inhibiting nitrification as compared with Baythroid. It is proposed that some microbial processes, nitrification in the present case, could be used as an indicator of persistence of some xenobiotics.

Key words: Nitrogen, Soil, Nitrification inhibitor

Introduction

Nitrogen (N) is quite often a limiting factor in crop production because of its relatively high requirements. Use of nitrogenous fertilizers has thus led to significant increases in crop yields. However, efficiency of fertilizer N use by plants is quite low as substantial amounts are lost from the soil-plant system through denitrification, NO_3 leaching and NH_3 volatilization (Broadbent 1965; Azam *et al* 1991; Lodhi *et al* 1996. Efforts have been and are being made to reduce N losses and increase the efficiency of fertilizer N use. Among several approaches adopted in this direction, use of nitrification inhibitors has met a fair degree of success in terms of increased crop yields and reduced N losses (Aulakh *et al* 1984; Webb *et al* 1991; Crawford and Chalk 1992). Studies have thus been conducted to find chemicals that selectively inhibit nitrification, a process with an important role in ecosystem functioning and having considerable environmental implications. Among the different nitrification inhibitors, N-Serve and DCD (Dicyandiamide) have been the most commonly used inhibitors. (Hauck 1980; Chalk *et al* 1990; Bronson *et al* 1991; Guiraud *et al* 1992). In addition, several insecticides have also been reported to have inhibitory effect on nitrification (Banerjee and Dey 1992; Das and Mukerjee 1994). Decrease in nitrifier population and rate of nitrification in the soil treated with insecticides has been reported (Martinez-Toledo *et al* 1992). Previously, we reported the effects of Baythroid (an insecticide produced by Bayer, Germany) on immobilization-remineralization of N (Lodhi *et al* 1994) and growth and N uptake of maize (Lodhi *et al* 1996). These studies demonstrated a significant positive effect of Baythroid on N cycling and growth of plants. In other studies, Baythroid was found to cause a positive change in soil microflora and some microbial processes (unpublished). In this paper we describe the results of experiments to: i) study the effect of Baythroid on nitrification and ii) compare Baythroid and N-serve (a well-known nitrification inhibitor) for inhibition of nitrification and persistence of the inhibitory effect.

*Author for correspondence.

Materials and Methods

Soil. Clay-loam soil was collected from the surface (0-15 cm) of an experimental field at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The physico-chemical properties of the air-dried and sieved (<2 mm) soil were as follows: organic C, 0.44%; total N, 0.05%; inorganic N (NH_4+NO_3+NO_2), 18.5 μg g^{-1}; soil pH (saturation extract), 7.8; sand, 19%; silt, 40%, clay, 41%.

Chemicals. Baythroid is the trade name of a synthetic pyrethroid manufactured by Bayer Chemicals, Germany and has cyfluthrin as an active ingredient. Baythroid is a commonly used insecticide on cotton in Pakistan. N-Serve or nitrapyrin is the most well known inhibitor of nitrification. Both the chemicals were of commercial grade and procured from the local market.

Experiment 1. Fifty g portions of the air-dried and sieved soil were taken in 100ml capacity plastic containers and treated with a solution of ammonium sulphate containing Baythroid to obtain final concentrations of 0.4, 1.6, and 6.4 $\mu g\ g^{-1}$ soil (on an active ingredient basis) and a moisture content of 55% WHC (water holding capacity). N-Serve was also used in aqueous solution to obtain final concentrations of 1.1, 4.4, and 17.6 $\mu g\ g^{-1}$ soil and a moisture content of 55% WHC. An untreated set was also incubated to serve as a control. In all the treatments, concentration of ammonium sulphate in aqueous solution was adjusted so as to get an N addition rate of 200 $\mu g\ g^{-1}$ soil and a moisture level equivalent to 55% WHC (water-holding capacity). The treated soil samples were incubated at 30°C in sufficient numbers to enable the removal of duplicate samples for the determination of NH_4-N and NO_3-N after different time intervals. Loss in moisture during incubation was made up by adding distilled water at alternate days. For analysis, 5 g soil samples were shaken for 1 h with 50 ml of 2*N* KCl solution and the suspension was filtered. The filtrate was analyzed for NH_4-N and NO_3-N by micro-Kjeldahl method (Bremmer and Keeney 1965).

Experiment 2. Most of the chemicals used as nitrification inhibitors loose their efficacy after certain period of time. This experiment was conducted to compare Baythroid and N-Serve for the persistence of their anti-nitrification activity over a period of 30 days. Fifty g portions of the soil were taken in plastic bottles and treated either with Baythroid at 0.4, 0.8, 1.6, 3.2, and 6.4 $\mu g\ g^{-1}$ soil or with N-Serve at 1.1, 2.2, 4.4, 8.8, and 17.6 $\mu g\ g^{-1}$ soil; both in water to obtain moisture content of 55% WHC. An untreated set was incubated to serve as a control. The treated soil samples were incubated at 30°C for 30 days at a constant moisture. Any loss in moisture was made up twice a week by adding distilled water. However, for the bottles to be used for studying the persistence of inhibitory (nitrification) effect of the test chemicals, addition of water to make up the moisture loss was stopped 3 days prior to second incubation phase with ammonium sulphate. This was considered necessary to allow for the addition of 1 ml ammonium sulphate solution without significantly disturbing soil moisture content. One ml of ammonium sulphate solution (containing enough N to obtain final N concentration in soil equivalent to 200 $\mu g\ g^{-1}$) was added to duplicate bottles from each treatment after 10, 20, and 30 days and the samples reincubated. After 10 days of reincubation, 5 g portions of soil from each bottle were extracted with 2*N* KCl solution (1:10, soil:solution ratio; 1 h shaking on a reciprocating shaker followed by filtration). The filtrates were analyzed for NH_4-N and NO_3-N separately by micro-Kjeldahl method (Brmner an Keeney 1965).

Results and Discussion

Table 1 shows the effect of Baythroid and N-Serve on the accumulation of NH_4-N and NO_3-N in soil samples after different incubation intervals. In untreated soil, NH_4-N disappeared fairly rapidly with negligible quantities after 1 week of incubation. At a Baythroid concentration of 0.4 $\mu g\ g^{-1}$ soil, substantial quantities of NH_4-N were determined after 1 week of incubation, while at 1.6 μg Baythroid g^{-1} soil, significant quantities (45.1 $\mu g\ g^{-1}$ soil) of NH_4-N were detectable even after 6 weeks of incubation. At highest levels of Baythroid tested, small decrease occurred in NH_4-N content after 1 week; the changes being still slower subsequently and after 7 weeks the soil contained 131.5 μg NH_4-N g^{-1} soil. In soil treated with N-Serve at 1.1. $\mu g\ g^{-1}$ soil, the decrease in NH_4-N content was quite obvious and consistent although much slower as compared with the lowest level of Baythroid used. At the two higher levels of N-Serve i.e. 4.4 and 17.6 $\mu g\ g^{-1}$ soil, the NH_4-N content of soil remained fairly constant after 1 week of incubation without showing any major decrease.

Trends in NO_3-N content of soil were generally opposite to that of NH_4-N. After 0.5 weeks, NO_3-N content varied between 13.4 and 20.4 $\mu g\ g^{-1}$ soil in different treatments; minimum NO_3-N content being recorded at the highest level of N-Serve. The content of NO_3-N increased fairly sharply and consistently in untreated soil and the one receiving lowest amount of Baythroid. However, the changes were fairly slow at increasing levels of both the inhibitors suggesting significant inhibition of nitrification.

Data on the percent inhibition of nitrification by the two inhibitors after 1, 3, 5, and 7 weeks of incubation is presented in Table 2. In all cases, N-Serve was found to cause a higher inhibition as compared with Baythroid. At the lowest level (0.4 $\mu g\ g^{-1}$ soil), Baythroid lost its inhibitory effect after 1 week of incubation, while at 1.6 $\mu g\ g^{-1}$, substantial inhibition (67.4%) was observed till 3rd week followed by a sharp decline. N-Serve showed stronger inhibitory effects even after 1 week of incubation and the effects persisted for extended period of incubation at all the three addition rates.

Persistence of inhibitory effects was demonstrated more clearly by the results of the second experiment (Table 3). As

Table 1

NH_4-N and NO_3-N content of soil samples incubated for different time intervals after treatment with different amounts of N-Serve and Baythroid.

Inhibitor, rate of addition µg g^{-1} soil	Weeks of incubation 0.5	1	2	3	4	5	6	7
	NH_4-N, µg g^{-1} soil							
Control	132.1	8.1	4.9	4.8	1.9	6.6	1.9	1.6
Baythroid 0.4	137.8	42.0	5.9	2.2	6.9	3.4	2.7	3.9
Baythroid 1.6	157.6	153.4	147.1	112.8	82.6	40.9	45.1	2.4
Baythroid 6.4	164.1	168.0	147.8	141.4	121.1	131.6	126.8	131.5
N-Serve, 1.1	154.0	123.0	79.8	60.4	48.7	40.9	46.6	38.4
N-Serve, 4.4	151.5	150.1	132.7	139.2	127.1	121.2	127.4	130.3
N-Serve, 17.6	148.7	150.9	142.2	139.4	139.4	145.2	140.6	145.8
	NO_3-N,µg g^{-1} soil							
Control	20.4	108.9	144.2	147.0	152.6	151.5	153.4	144.5
Baythroid 0.4	20.1	83.4	133.6	140.3	142.9	150.5	150.8	151.8
Baythroid 1.6	16.0	25.8	33.3	47.9	79.7	123.2	128.8	142.8
Baythroid 6.4	10.4	16.4	20.4	30.0	43.7	44.8	47.3	51.0
N-Serve, 1.1	17.4	45.9	76.4	102.8	108.5	127.9	93.5	104.2
N-Serve, 4.4	13.6	16.4	18.5	22.1	28.2	29.7	32.5	35.3
N-Serve, 17.6	13.4	14.2	12.9	14.0	14.0	17.1	17.4	20.7
Overall LSD (P=0.05), 4.9								

expected, extent of nitrification inhibition increased consistently with the rate of the two inhibitors. After 10 days of incubation, the two inhibitors caused fairly similar inhibition of nitrification except at the two lower levels of addition, where N-Serve was more inhibitory. The differences in the extent of inhibition became increasingly distinct after 20 and 30 days of incubation prior to addition of NH_4-N. With the passage of time, inhibitory effects subsided in all treatments. However, the decrease in inhibitory effects with time was more pronounced in case of Baythroid (% inhibition at the highest level of Baythroid decreasing from 88.1after 10 days to 69.0 after 30 days; respective values for N-Serve were 89.3 and 86.2), suggesting greater persistence of N-Serve as a nitrification inhibitor.

In order to conserve fertilizer N by limiting NO_3 leaching and denitrification losses of nitrate from soil, nitrification inhibitors like N-Serve have shown immense promise. The inhibitors generally block oxidation of NH_4 resulting in its accumulation (Bremner and Blacker 1978, Guiraud *et al* 1989). NH_4-N accumulating in soil as a result of nitrification inhibition can be adsorbed to the soil exchange sites resulting in temporary or prolonged conservation of N through reduced NH_3-volatilization losses. Further, NH_4-N is immobilized/assimilated by the soil microbes in preference to NO_3 (Jansson 1958). Nitrification inhibition may, therefore, be advantageous for the microbial growth and activities. In addition, NH_4-N is reported to enhance the mineralization of native soil N leading to its increased availability to plants (Azam *et al* 1991, 1995, Jenkinson *et al* 1985). In a previous study, Baythroid was also found to accelerate the process of N mineralization (Lodhi *et al* 1994). Plants are reported to be less competitive with soil microorganisms for NH_4^+ (Schimel *et al* 1989). Consequently, higher amounts of NO_3^- will be available for plant uptake following inhibition of nitrification.

In the present study also, N-Serve caused a significant and persistent inhibition of nitrification. Inhibition of nitrification by N-Serve has been demonstrated in many studies (Hauck 1980, Chalk *et al* 1990, Bronson *et al* 1991, Guiraud *et al* 1992). In most of these studies, N-Serve has been applied at 1.1 µg g^{-1} soil, whereas we have studied nitrification at this level as well as 4 and 16 times the level generally used. The inhibitory effects were found to be fairly persistent especially at higher rates of N-Serve. Application of Baythroid also lead to a significant inhibition of nitrification in soil. Inhibition of nitrification has been demonstrated for pesticides including insecticides and fungicides e.g. Truban and Captan have been reported to inhibit nitrification substantially (Feng and Barker 1990).

In the present study, rate of Baythroid addition varied from 0.4 to 6.4 µg g^{-1} soil with higher rates causing substantial and persistent inhibition of nitrification. Some other authors have used amounts of insecticides ranging between 10 and 300 µg g^{-1} soil (Martinez Toledo *et al* 1992). In other studies, significant side effects of sequentially and simultaneously applied pesticides on non-target soil microbes in laboratory experiments have been reported (Schuster and Schroeder 1990). These studies showed that nitrification was highly altered upon overdosage and turnover completely stopped indicating that specific functions of microflora may be completely arrested. Inhibition of nitrification by heterocyclic compounds containing two or three adjacent ring N atoms (as in cyfluthrin) has been reported (McCarty and Bremner 1989). In addition, several insecticides have also been reported to have inhibitory effect on nitrification (Banerjee and Dey 1992, Das and Mukrjee 1994). Decrease in nitrifier population and rate of nitrification in soil treated with insecticides has been reported (Martinez - Toledo *et al* 1992).

The inhibitory effect of Baythroid was relatively less persistent as compared with N-Serve particularly at lower rates of application which may suggest a rapid binding onto the soil exchange sites and presence of still lower amounts in forms effective against nitrifiers. This assumption is supported by the recommendations for application of higher doses of special purpose chemicals to soils high in binding sites i.e., the soils with high organic matter and clay content. In the present study the inhibitory effect of both Baythroid and N-Serve persisted for longer period at higher rates of application, an observation that supports the preceding assumption. The results of this study also suggest that the fate and persistence of an agrochemical may be determined indirectly through its effect on certain soil microbial processes e.g., nitrification inhibition in the case of Baythroid.

Table 2

Percent inhibition of nitrification by the two inhibitors at some selected incubation intervals.

Inhibitor, rate of addition (µg g^{-1} soil)	Weeks of incubation 1	3	5	7
	Percent inhibition of nitrification			
Baythroid 0.4	23.4	4.6	0.3	-5.1
Baythroid 1.6	76.3	67.4	18.4	1.2
Baythroid 6.4	84.9	79.6	70.3	64.7
N-Serve, 1.1	57.9	30.1	15.3	27.9
N-Serve, 4.4	84.9	85.0	80.3	75.6
N-Serve, 17.6	87.0	90.5	88.7	85.7
Overall LSD (P=0.05), 4.4				

Table 3

Persistence of N-Serve and Baythroid as nitrification inhibitors after different residence time in soil

Inhibitor, rate of addition (µg g^{-1} soil)	Days of incubation in soil 10	20	30
	Percent inhibition of nitrification		
Baythroid 0.4	21.9	10.2	7.6
Baythroid 0.8	61.2	20.1	23.5
Baythroid 1.6	82.9	23.3	23.9
Baythroid 3.2	87.1	76.6	32.2
Baythroid 6.4	88.1	84.8	69.0
N-Serve, 1.1	55.3	3.9	1.5
N-Serve, 2.2	74.7	11.1	15.0
N-Serve, 4.4	87.7	73.6	32.3
N-Serve, 8.8	89.1	87.0	80.3
N-Serve, 17.6	89.3	89.3	86.2
Overall LSD (P=0.05), 5.2			

References

Aulakh M S, Rennie DA, Paul E A 1984 Acetylene an N-serve effects upon N_2O emissions from NH_4 and NO_3 treated soils under aerobic and anaerobic conditions. *Soil Biol Biochem* **16** 351-356.

Azam F, Lodhi A, Ashraf M 1991 Interaction of ^{15}N-labelled ammonium nitrogen with native soil nitrogen during incubation and growth of maize (*Zea mays* L). *Soil Biol Biochem* **23** 473-477.

Azam F, Mulvaney R L, Simmons F W 1995 Effects of ammonium and nitrate on mineralization of N from leguminous residues. *Biol Fertil Soils* **20** 49-52.

Banerjee M R, Dey B K 1992 Effects of different pesticides on microbial population nitrogen mineralisation and thiosulphate oxidation in the rhizosphere of jute (*Corchorus capsularis* L cv). *Biol Fertil Soils* **14** 213-218.

Bremner J M, Keeney K R 1965. Steam distillation method for determination of ammonium nitrate and nitrite *Anal Chem Acta* **32** 485-495.

Bremner J M, Blackmer A M 1978 Nitrous oxide emissions from soils during nitrification of fertilizer nitrogen. *Science* **199** 295-296.

Broadbent F E 1965 Effect of fertilizer nitrogen on the release of soil nitrogen. *Soil Sci Soc Am Proc* **29** 692-696.

Bronson J M, Touchton J T, Hauck R D, Kelley K R 1991 Nitrogen-15 recovery in winter wheat as affected by application timing and dicyandiamide. *Soil Sci Soc Am J* **55** 130-135.

Chalk P M, Victoria R L, Muraoka T, Piccolo M C 1990 Effect of nitrification nihibitor on immobilization and mineralization of soil and fertilizer nitrogen. *Soil Biol Biochem* **22** 533-538.

Crawford D M, Chalk P M 1992 Sources of N uptake by wheat (*Triticum aestivum* L) and N transformations in soil treated with a nitrification nihibitor. *Plant and Soil* **149** 59-72.

Das A C, Mukherjee D 1994 Effect of insecticides on the availability of nutrients, nitrogen fixation and phosphate solubility in the rhizosphere of rice. *Biol Fertil Soils* **18** 37-41.

Feng J, Barker A B 1990 Growth and mineral composition of radish under different nitrification inhibitors and nitrogen sources. *Commun Soil Sci Plant Anal* **20** 623-633.

Guiraud G, Marol C, Thibaud M C 1989 Mineralization of nitrogen in the presence of a nitrification inhibitor. *Soil Biol Biochem* **21** 29-34.

Guiraud G, Marol C, Fardeau J C 1992 Balance and immobilization of $(^{15}NH_4)_2SO_4$ in a soil after the addition of Didin as a nitrification inhibitor. *Biol Fertil Soils* **14** 23-29.

Hauck R D 1980 Mode of action of nitrification inhibitors In: *Nitrification inhibitors Potentials and Limitations,* eds Meisinger J J *et al*, Spec Publ 38, Am Soc Agron, Madison, Wisconsin, pp 19-32.

Jansson S L 1958 Tracer studies on nitrogen transformations in soil with special attention to mineralisation - immobilization relationships. *Ann R Agric Coll Swed* **24** 101-361.

Jenkinson D S, Fox R H, Rayner J H 1985 Interactions between fertilizer nitrogen and soil nitrogen -- the so-called "priming" effect. *J Soil Sci* **36** 425-444.

Lodhi A, Malik N N, Azam F 1994 Effect of baythroid on nitrogen transformations in soil. *Biol Fertil Soils* **17** 173-176.

Lodhi A, Malik N N, Azam F 1996 Growth and nitrogen nutrition of rice (*Oryza sativa* L) in soil treated with N-serve an nitrification inhibiting insecticide. *Pak J Bot* **28** 75-83.

Martinez-Toledo M V, Salmeron V, Gonzalez-Lopez J 1992 Effect of the insecticide methylpyrimifos and chlorpyrifos on soil microflora in a agricultural loam. *Plant and Soil* **147** 25-30.

McCarty G W, Bremner J M 1989 Inhibition of nitrifaction in soil by heterocyclic compounds. *Biol Fertil Soils* **8** 204-211.

Schimel J P, Jackson L E, Firestone M K 1989 Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. *Soil Biol Biochem* **21** 1059-1066.

Schuster E, Schroeder D 1990 Side-effects of sequentially-applied pesticides on non-target soil microorganisms. Field experiments. *Soil Biol Biochem* **22** 367-373.

Webb J, Froment M, Sylvester-Bradley R 1991 Effects of cultivation and nitrification inhibitor on soil nitrogen availability after a grass ley and on the response of the following cereal crop to fertilizer nitrogen. *J Agric Sci Camb* **117** 9-21.