

## GROWTH AND NITROGEN NUTRITION OF RICE (*ORYZA SATIVA* L.) IN SOIL TREATED WITH N-SERVE AND A NITRIFICATION INHIBITING INSECTICIDE

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### Abstract

A pot experiment was conducted to study the effect of N-Serve (nitrification inhibitor) and Baythroid (an insecticide with strong inhibitory effect on nitrification in soil) on dry matter yield and N uptake of rice (*Oryza sativa* L.). N-Serve was applied @ 1.1, 4.4 mg kg<sup>-1</sup> soil and Baythroid @ 1.6 and 6.4 mg kg<sup>-1</sup> soil, either alone or together with <sup>15</sup>N-labelled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> @ 200 mg kg<sup>-1</sup> soil. Both N-Serve and Baythroid caused an increase in the dry matter yield of root and shoot portions, with significant decrease in the grain weight. There was no significant effect of either inhibitor on total dry matter yield and N uptake. Nitrogen yield and dry matter yield were significantly correlated in different treatments.

Different treatments exerted a similar effect on total N as observed for dry matter yield. Efficiency of N fertilizer uptake varied from 49 to 59% in different treatments. Different method gave relatively higher values for the fertilizer use efficiency as compared to isotopic method. Only 11-16% of applied N was recovered in soil after harvesting the plants and both the inhibitors caused a higher incorporation of applied N into soil organic matter. Upto 30-43% of applied N was lost from the soil-plant system with more losses from soil treated with N-Serve. Application of N fertilizer caused a significant increase in N uptake from native soil organic matter suggesting the occurrence of a positive added nitrogen interaction (ANI). N-Serve lead to a decrease in the ANI, while Baythroid had a significantly positive effect at the lower level of application.

### Introduction

Use of nitrogenous fertilizers has led to significant improvement in crop yields. However, low N use efficiency of plants has been of concern because of economic as well as environmental reasons. The problem of low N use efficiency is particularly serious in flooded rice ecosystem where the plants are reported to assimilate only 30-50% of the applied N fertilizer while a significant component is lost from the soil-plant system (Craswell & Vlek, 1979; Prasad & De Datta, 1979). The low use efficiency of rice is attributed to heavy losses of applied N mainly through denitrification (De Datta, 1986) in spite of the fact that nitrification will be fairly inhibited under submerged conditions. However, rice plant is able to transport oxygen into the rhizosphere through aerenchyma that may facilitate nitrification. In addition, nitrification is reported to occur from the upper oxidized layer. Formation of NO<sub>3</sub> and its mobility to anaerobic soil zones could thus lead to substantial loss through denitrification as well as escape from the effective zones of root uptake. Use of nitrification inhibitors may therefore be an appropriate approach to decrease these losses and improve fertilizer use efficiency and plant growth. Several studies have shown a positive effect of nitrification inhibitors on N dynamics in soil, N availability to plants and plant growth (Chalk *et al.*, 1990;

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Clay *et al.*, 1990; Walters & Malzer, 1990; Bronson *et al.*, 1991; Guiraud *et al.*, 1992). In most of these studies, crops other than rice have been used. In rice, however, use of nitrification inhibitors is not very common and the little available data show variable effects of inhibitors on grain yield (Prasad & Power, 1995)

Among the different nitrification inhibitors, N-Serve and DCD have been most commonly used (Reddy, 1963; Hauck, 1980; Chalk *et al.*, 1990; Walters & Malzer, 1990; Bronson *et al.*, 1991; Guiraud *et al.*, 1992). Besides, several insecticides are also reported to have inhibitory effect on nitrification (Banerjee & Dey, 1992; Das & Mukherjee, 1994). During our studies, we have observed that Baythroid (an insecticide) leads to a strong inhibition of nitrification in soil (Lodhi *et al.*, 1994). It also accelerates the mineralization of organic N and immobilization-mineralization turnover of inorganic N in the presence of an easily oxidizable carbon source. These effects are different from N-Serve and DCD which are generally reported to have no effect on soil microbial processes other than nitrification (Guiraud *et al.*, 1989). Baythroid was thus found to cause a significant increase in the dry matter yield in maize (Lodhi *et al.*, 1996). This increase was found due mainly to an increased uptake by plants of N from applied as well as native soil N. The increase in N uptake from soil was attributed to the added nitrogen interaction resulting from prolonged availability in soil of  $\text{NH}_4\text{-N}$  and from enhanced mineralization of native soil organic N. The objective of the present investigation was to see if similar effects are observed under submerged conditions using rice (*Oryza sativa* L.) as a test crop.

## Materials and methods

### Soil

The soil used in this study was collected from the surface (0-15 cm) of an experimental field at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Physico-chemical analyses of the air-dried and sieved (<2 mm) soil showed organic C content 0.44%; total N 0.05%; inorganic N ( $\text{NH}_4 + \text{NO}_3 + \text{NO}_2$ ), 18.5 mg  $\text{kg}^{-1}$  soil; pH, 7.8; sand 19%; silt 40% and clay 41%. Analytical methods used have been described by Azam *et al.*, (1994).

### Nitrification inhibitors

Baythroid (cyfluthrin) a synthetic pyrethroid having the chemical formula: Cyano-(4-fluoro-3-phenoxyphenyl)-methyl-3-(2,2-dichlorophenyl)-2,2-dimethyl-cyclopropanecarboxylate manufactured by Bayer Leverkusen, Germany was used. It is a highly effective and fast-acting insecticide with long lasting residual activity which acts mainly as a contact poison. The uniformity of Baythroid in solution was ascertained spectrophotometrically.

N-Serve, trade name of Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] a well known nitrification inhibitor, was used as an aqueous solution.

### Pot experiment

Five-kg portions of air-dried and sieved (< 2 mm) soil was filled in 6-kg plastic pots. Nitrogen as  $^{15}\text{N}$ -labelled  $(\text{NH}_4)_2\text{SO}_4$  (3.0 atom %  $^{15}\text{N}$ ) was applied in solution form @ 50, 75, and 75 mg  $\text{Kg}^{-1}$  at transplanting, tillering and anthesis, respectively. N source @ 1.1 and 4.4 mg  $\text{Kg}^{-1}$  and Bathyroid @ 1.6 and 6.4 mg  $\text{Kg}^{-1}$  with and without N @ 200 mg  $\text{Kg}^{-1}$  soil was used. All pots, including control pots, received P and K @

**Table 1. Dry matter yield of rice and its distribution in different plant components as affected by different soil treatments.**

Treatments	Dry matter yield (g pot <sup>-1</sup> )				Harvest index
	Root	Straw	Grain	Total	
Control	7.1c	21.0d	12.7d	40.8c	0.38c
N, 200 mg kg <sup>-1</sup>	15.2b	46.2b	42.6a	104.0b	0.48a
N-Serve, 1.1 mg kg <sup>-1</sup> soil	7.1c	21.4d	11.7e	40.2c	0.35cd
N-Serve, 4.4 mg kg <sup>-1</sup> soil	7.0c	20.9d	12.4d	40.3c	0.37cd
N-Serve 1.1 mg and N, 200 mg kg <sup>-1</sup>	18.3a	54.6a	30.8c	103.7b	0.36cd
N-Serve 4.4 mg and N, 200 mg kg <sup>-1</sup>	18.1a	46.8b	34.1b	99.0b	0.42bc
Baythroid, 1.6 mg kg <sup>-1</sup>	7.5c	21.0d	13.2d	41.7c	0.39c
Baythroid, 6.4 mg kg <sup>-1</sup>	7.5c	25.9c	10.0e	43.4c	0.28d
Baythroid 1.6 mg and N, 200 mg kg <sup>-1</sup>	20.3a	53.3a	44.4a	118.0a	0.45ab
Baythroid 6.4 mg and N, 200 mg kg <sup>-1</sup>	15.2b	57.6a	32.4bc	105.2a	0.36cd

Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability according to Duncan's Multiple Range Test.

50 and 62 mg Kg<sup>-1</sup>, respectively as KH<sub>2</sub>PO<sub>4</sub> just before transplanting. Three one-month old seedlings of rice (*Oryza sativa* L., cv IR-6) were transplanted per pot in July 1994 and the plants grown to maturity under flooded conditions. At harvest, data on dry matter of roots, straw and grains was collected and aliquots of the finely powdered material were analyzed for Kjeldahl N (Bremner & Mulvaney 1982). Sub-samples of sieved soil were also analyzed for total N (including NO<sub>3</sub>-N). After titration, the distillates were processed for isotope-ratio analyses on a mass spectrometer (Varian MAT GD 150).

## Results

Dry matter in the three plant components was affected similarly by different treatments as suggested by significant correlation when dry matter of any two components was compared (Table 1). Application of N fertilizer had a significant positive effect on dry matter of all three plant components. Application of both N-Serve and Baythroid in the absence of N fertilizer had no significant effect on dry matter yield of different plant components irrespective of the amount applied. In the presence of N, both inhibitors had somewhat positive effect on dry matter of root and shoot components although rate of application had no consistent effects. Grain yield was significantly reduced in the presence of N-Serve at both levels of application. Baythroid, on the other hand, caused a small but non-significant increase at lower level and a significant decrease at higher rate of application. Apparently, the two nitrification inhibitors affected the distribution of dry matter among grain and straw with non-significant effects on total dry matter yield except at lower level of Baythroid that

**Table 2. Nitrogen yield of rice and its distribution in different plant components as affected by different soil treatments.**

Treatments	Nitrogen yield (mg pot <sup>-1</sup> )				Harvest index
	Root	Straw	Grain	Total	
Control	41.2e	66.8f	118.1d	226.1e	0.64b
N, 200 mg kg <sup>-1</sup>	92.3c	190.3c	582.0a	864.6ab	0.75a
N-Serve, 1.1 mg kg <sup>-1</sup> soil	36.9f	84.6e	106.8d	228.3e	0.56c
N-Serve, 4.4 mg kg <sup>-1</sup> soil	34.3f	70.6f	129.8cd	234.7e	0.65b
N-Serve 1.1 mg and N, 200 mg kg <sup>-1</sup>	119.5b	226.6b	419.9b	766.0c	0.65b
N-Serve 4.4 mg and N, 200 mg kg <sup>-1</sup>	117.0b	182.8c	449.8b	749.6c	0.60bc
Baythroid, 1.6 mg kg <sup>-1</sup>	40.0e	73.1f	136.8c	249.1de	0.65b
Baythroid, 6.4 mg kg <sup>-1</sup>	50.7d	112.1d	115.8d	278.6d	0.51c
Baythroid 1.6 mg and N, 200 mg kg <sup>-1</sup>	142.3a	244.0b	576.4a	962.7a	0.70ab
Baythroid 6.4 mg and N, 200 mg kg <sup>-1</sup>	111.0b	282.1a	420.8b	813.9b	0.60bc

Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability according to Duncan's Multiple Range Test.

caused a significant increase. Both N-Serve and Baythroid caused a substantial decrease in the harvest index suggesting that the two inhibitors caused significant changes in dry matter partitioning.

Trends in nitrogen yield (Table 2) were fairly similar to those observed for dry matter yield in different treatments with maximum N yield (142.3 mg pot<sup>-1</sup>) and minimum (34.3 mg pot<sup>-1</sup>) were recorded. Consequently, N yield of root, straw and grain portions showed a close relationship with dry matter yield of the respective components; coefficient of correlation being 0.98, 0.98 and 0.99, respectively. This would suggest the dependence of dry matter yield on N uptake. Similarly, close relationship ( $r = 0.88$ ) was also observed between harvest index (HI) and N harvest index (NHI) in different treatments. Nitrogen content of the three plant components was affected by different treatments similar to that noted for dry matter. Contribution of fertilizer to the total N content of different plant components varied between 51% and 72%; higher values being recorded for grain portion in all the treatments (Table 3). The treatment differences in terms of percent contribution of applied N to the total N of any component were generally non significant. All components taken together showed fertilizer contribution of 61-65%, the differences between treatments being non significant.

The two inhibitors had generally a negative effect on uptake of fertilizer N except at the lower level of Baythroid (Table 4). In the absence of inhibitors, rice plants were found to use 56% of the applied N, while in the presence of inhibitors, the values varied between 44 and 59%; the lowest being for N-serve and highest for Baythroid. Both the inhibitors caused a significant increase in the fertilizer N remaining in soil (immobilized by soil microflora or deposited in the soil as plant residues). Only at

lower level of Baythroid, losses of applied N were reduced to some extent, while in other treatments, the losses were more as compared to control.

Application of fertilizer N increased the availability of unlabelled N (derived from soil organic matter or through biological nitrogen fixation) to plants as suggested by higher amount of N derived from soil; the amount of soil N in plants being 302.7 mg pot<sup>-1</sup> from fertilized compared to 226.1 mg pot<sup>-1</sup> from unfertilized soil (Table 4). Application of N-Serve at either level, with or without fertilizer N, had no significant effect on the availability to plants of soil N. Baythroid, on the other hand, increased the uptake of soil N from 226.1 mg pot<sup>-1</sup> to 371.9 mg pot<sup>-1</sup> and 307.6 mg pot<sup>-1</sup> at lower and higher level, respectively; the increase being significant at higher level of application in the presence of N fertilizer and reverse was true in the absence of fertilizer. The changes in the uptake of soil N due to N fertilizer and the inhibitors were exhibited as added nitrogen interaction (ANI), both the inhibitors leading to ANIs.

## Discussion

Nitrification inhibitors are reported to increase crop yield mainly by prolonging the availability of NH<sub>4</sub> in soil leading to decreased losses of N through denitrification and NO<sub>3</sub> leaching and thus enhancing the N use efficiency of plants (Walters & Malzer, 1990). An added benefit of prolonged availability of NH<sub>4</sub> in soil is its preferential assimilation by microbes and thereby enhanced overall microbial activities including mineralization of native soil N (Jenkinson *et al.*, 1985; Azam *et al.*, 1995). In addition, nitrification inhibition is reported to increase microbial immobilization of applied fertilizer N thereby conserving it for subsequent release and use by plants (Clay *et al.*, 1990; Crawford & Chalk, 1993). However, both positive and negative effects of nitrification inhibitors on plant growth have been reported (Hauck, 1980; Clay *et al.*, 1990; Walters & Malzer, 1990). In the present studies, both N-Serve and Baythroid were found to exert a negative or a positive but nonsignificant effect on total N content of the plants (Table 3). Patrick *et al.*, (1968) also reported no advantage of N-Serve for rice, while Wells (1976) showed an increase in rice grain yield. Since dry matter yield is directly related to plant N uptake, this effect was reflected on dry matter yield of rice which was not positively influenced by the use of nitrification inhibitors. To some extent, Baythroid enhanced the uptake of N leading to increased dry matter production. It is interesting to note that Baythroid was not as effective for rice as observed for maize (Lodhi *et al.*, 1996). However, both N-Serve and Baythroid caused a significant increase in N content and dry matter yield of root and straw components at the expense of grain portion. Apparently, the inhibitors affected the partitioning of nutrients e.g., N within the plant rather than inhibiting the uptake.

Contrary to expected effects, neither of the nitrification inhibitors had a significantly positive effect on N fertilizer use by plants (Table 3 & 4) and only Baythroid was effective to some extent in reducing the N fertilizer losses. It would appear therefore that losses of N occurred mainly through NH<sub>3</sub> volatilization and since both the inhibitors would prolong the availability of NH<sub>4</sub> in soil, this could lead to higher fertilizer N losses. Indeed, substantial losses of NH<sub>3</sub>-N from flooded rice ecosystem have been reported (Mikkelsen & De Datta, 1979). Under these conditions,

**Table 3. Plant N derived from fertilizer and its distribution in different components of rice.**

Treatments	N derived from fertilizer (mg pot <sup>-1</sup> )			
	Root	Straw	Grain	Total
N, 200 mg kg <sup>-1</sup>	51.2d (51.4)*	97.8c (51.4)	413.0a (71.0)	562.0a (65.0)
N-Serve 1.1 mg and N, 200 mg kg <sup>-1</sup>	68.2b (51.01)	123.3b (54.4)	301.6b (71.8)	493.1b (64.4)
N-Serve 4.4 mg and N, 200 mg kg <sup>-1</sup>	64.7bc (55.2)	100.4bc (5.9)	290.9b (72.3)	456.0c (60.8)
Baythroid, 6.4 mg kg <sup>-1</sup>	81.9a (57.5)	115.6b (47.4)	393.3a (68.2)	590.8a (61.4)
Baythroid 1.6 mg and N, 200 mg kg <sup>-1</sup>	60.7c (54.7)	145.4a (51.5)	300.2b (71.3)	506.3b (62.2)

\*Figures in parentheses indicate percent N derived from fertilizer. Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability according to Duncan's Multiple Range Test.

enhanced losses through NH<sub>3</sub> volatilization have been attributed to increase in pH of the flood water due to NH<sub>4</sub> (Vlek & Craswell, 1981). In the present study, 30-43% of the applied N was unaccounted in the soil-plant system. The values are within the range generally reported for the lowland rice (Crasswell & Vlek, 1979). Nevertheless, the unaccounted N calculated as a difference of N fertilizer initially applied and that recovered in the soil-plant system could have been over-estimated since substantial quantities of NH<sub>4</sub> could be fixed in soil and could not be determined by the normal Kjeldahl method used in this study. The nitrification inhibitors would add to this problem by prolonging the availability of NH<sub>4</sub>-N.

One of the attributes of nitrification inhibitors is the enhanced immobilization of N in soil organic matter. In the present study, significantly higher quantities of fertilizer N were determined in soil treated with N-Serve and Baythroid compared to control following plant harvest (Table 4). Enhanced N immobilization in the presence of nitrification inhibitors is reported to be one of the reasons for reduced plant N uptake (Clay *et al.*, 1990). In the present study, however, because of the lower residual amounts in soil, the overall impact of enhanced immobilization on total N fertilizer N balance may not appear substantial. For N-Serve, positive as well as negative and no effects on N immobilization have been reported depending upon the incubation conditions and availability of carbon (Aulakh & Rennie, 1984; Myrold & Tiedje 1986; Clay *et al.*, 1990). In a soil-plant system, however, carbon supply in the form of rhizodeposits may be substantial to cause an increase in the immobilization of fertilizer N in the presence of a nitrification inhibitor and an increase in net immobilization of N could be expected as reported by Clay *et al.*, (1990). Baythroid has previously been shown to cause a significant increase in immobilization of N as well as remineralization

**Table 4. Fate of applied fertilizer N in soil-plant system, uptake of soil N by rice plants and the ANI.**

Treatments	Fertilizer N (% of applied)			Soil N (mg pot <sup>-1</sup> )	
	Plant	Soil	Unaccounted	Plant	ANI
Control	---	---	---	226.1d	---
N, 200 mg kg <sup>-1</sup>	56.2ab	9.7c	34.1b	302.7bc	6.6c
N-Serve, 1.1 mg kg <sup>-1</sup> soil	---	---	---	228.3d	2.2f
N-Serve, 4.4 mg kg <sup>-1</sup> soil	---	---	---	234.7d	8.6f
N-Serve 1.1 mg and N, 200 mg kg <sup>-1</sup>	44.3c	12.6a	43.1a	322.9b	96.8b
N-Serve 4.4 mg and N, 200 mg kg <sup>-1</sup>	49.1bc	12.0ab	38.9ab	299.1bc	73.0c
Baythroid, 1.6 mg kg <sup>-1</sup>	---	---	---	249.1d	23.0e
Baythroid, 6.4 mg kg <sup>-1</sup>	---	---	---	278.6c	52.5d
Baythroid 1.6 mg and N, 200 mg kg <sup>-1</sup>	59.1a	11.2c	29.7c	371.9a	145.8a
Baythroid 6.4 mg and N, 200 mg kg <sup>-1</sup>	51.0b	12.9b	36.1b	307.6bc	81.5c

Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability according to Duncan's Multiple Range Test.

of immobilized N (Lodhi *et al.*, 1994). Similar effects have been reported for some other insecticides (Banerjee & Dey, 1992) which are attributed to enhance availability of NH<sub>4</sub> to soil microorganisms.

Application of inorganic N is reported to cause an increase in the mineralization of native soil N as a result of the added nitrogen interaction or ANI (Jenkinson *et al.*, 1985; Hart *et al.*, 1986; Chalk *et al.*, 1990; Azam *et al.*, 1995; Lodhi *et al.*, 1996). The ANI is reportedly higher in the presence of NH<sub>4</sub> than NO<sub>3</sub> (Hart *et al.*, 1986; Azam *et al.*, 1995). Nitrification inhibition would thus lead to a higher ANI (Azam *et al.*, 1995). In the present study, fertilized soil showed significantly higher amounts of soil N in plant and both N-Serve and Baythroid had a significantly additive effect, the latter being more effective. Even in the absence of fertilizer N, substantially higher quantities of soil N were made available to plants due to Baythroid, particularly at lower levels of application and higher rates of Baythroid seemed to be detrimental to soil microflora as well as to plants. Although nitrification inhibition potential of N-Serve and Baythroid was found to be fairly similar in a previous study (Lodhi *et al.*, 1994), the two inhibitors exhibited significantly different levels of ANI. This may be attributed to almost negligible effects of N-Serve on microbial activities other than nitrification (Guiraud *et al.*, 1989; Crawford & Chalk, 1993) compared to an overall positive effect of Baythroid (Lodhi *et al.*, 1994).

Results of the present study suggest that nitrification inhibitors may not have a great value for N economy and productivity of lowland rice ecosystems. This observation is in contrast to the results reported by Wells (1976) but agrees with those reported by Touchton & Boswell (1980) and Sudakhara & Prasad (1986). The present studies do suggest, however, that N availability to rice from sources other than the commercial fertilizer could be increased by using nitrification inhibitors. More studies are necessary to clearly elucidate the role of nitrification inhibitors in rice ecosystem.

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