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ISSN 0792 - 156X

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PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

USE OF NITRIFICATION INHIBITORS TO INCREASE EFFICIENCY OF NITROGENOUS FERTILIZERS IN AQUACULTURE

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(Received 27.1.04, Accepted 9.6.04)

Key words: nitrogen fertilization, nitrification inhibitors, primary productivity

Abstract

High doses of nitrogenous fertilizers are often applied in fishponds but only a small part of the added nitrogen is utilized for improving primary productivity and, thereby, fish yields. A large portion of the nitrogen is lost from the pond environment through various mechanisms, especially nitrification. In the present investigation, we studied the effects of three nitrification inhibitors: (a) neem *(Azadirachta indica)* extract, (b) karanj *(Pongamia glabra)* extract, and (c) sodium azide (NaN_3), on the transformation of applied nitrogen in simulated fishpond conditions. The study revealed that nitrification inhibitors considerably retard the rate of nitrification from the ammonium form of nitrogen (NH_4^+), resulting in a higher amount of nitrogen in the soil and water. The increased nitrogen concentration significantly increased gross primary production and, hence, improved the efficiency of the added nitrogen.

Introduction

Nitrogenous fertilizers are applied to fishponds to increase primary productivity at rates as high as 200-400 kg/ha (Boyd et al., 2002). However, only a small portion of the added nutrient is actually utilized for primary production, and thereby fish culture, while a large share is lost by various means including volatilization, leaching, and denitrification (Bouldin et al., 1974; Chattopadhyay and De, 1991). Schroeder (1987), while working on the nitrogen budget of a fishpond, observed that only 18% of the total nitrogen added as manure and fertilizer was incorporated into fish flesh while 52% was lost through various mechanisms. Gross et al. (2002) observed that in channel catfish ponds only 31.5% of the available nitrogen was ultimately transmitted into fish flesh and a large amount of the

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nutrient was lost through denitrification, leaching, and volatilization.

This large-scale loss not only increases aquaculture costs but may also affect the quality of ground water through leaching of NO_3^-. Mandal and Chattopadhyay (1992) suggested that maintaining a higher amount of NH_4^+-N than NO_3^--N in the pond environment may increase efficiency of nitrogenous fertilizers in fishponds. Since NH_4^+ can be adsorbed by soil colloids in an easily exchangeable phase, loss of this form of nitrogen will be reduced.

Compared to agricultural soils, the presence of high water tables in fishponds is likely to reduce the volatilization loss of NH_4^+. Mandal (1984) suggested using nitrification inhibitors to maintain a higher nitrogen content in submerged rice soils by restricting the release of NO_3^--N from commonly used nitrogenous fertilizers. Despite the ecological similarity between fishponds and submerged rice soils (Hickling, 1971), studies on nitrification inhibitors in fishponds are lacking. In this investigation, we studied the effects of three nitrification inhibitors in conjunction with a commonly used nitrogenous fertilizer, i.e., urea, and primary productivity.

Materials and Methods

Bottom soil was collected from a fishpond in a red and lateritic soil zone of West Bengal, India. The soil was air dried, sieved through a 2 mm and then 80 mesh sieve, analyzed, and placed in 36 glass aquaria of 28 liters (30.5 x 30.5 x 30.5 cm) at 1 kg soil per aquarium. The soil was submerged in de-ionized water at a soil:water ratio of 1:15 and left for 10 days to develop simulated semi-aerobic fishpond conditions, described as a microcosm by Boyd and Munsiri (1997).

Nitrogen, at a dose of 0, 50, or 100 mg/kg soil, was applied to the aquaria in the form of urea. For each dose, there were four treatments: (a) no nitrification inhibitor, (b) neem (*Azadirachta indica*) extract, (c) karanj (*Pongamia glabra*) extract, and (d) sodium azide (NaN_3). Each treatment was replicated thrice. The nitrification inhibitor was mixed with urea at 1%. In addition, each treatment received phosphorus and potassium at 50 and 15 mg/kg soil, respectively, to minimize any limiting effect of a possible deficiency of these major nutrients on the growth of primary fish food organisms.

The aquaria were incubated at an average 340 lux. The soil and water of each aquarium were periodically sampled and analyzed for easily mineralizable nitrogen (Subbiah and Asija, 1956). An acidified NaCl solution (Jackson, 1973) was used to extract the NH_4^+-N and NO_3^--N from the soil. After estimating the amount of NH_4^+-N in the samples, the amount of NO_3^--N was determined using Devarday's alloy (Jackson, 1973). In each case, nitrogen was determined with a semi-automatic Kjeltec apparatus. Gross and net primary productivity of the water were determined by the dark and light bottle method (Odum, 1973). Variations between treatments were analyzed statistically by determining the critical difference. Mean treatment values were used for Duncan's multiple range test.

Another microcosm study was carried out to assess the magnitude of NH_3 lost by volatilization. Pond soils were incubated with 1000 ml de-ionized water at a soil:water ratio of 1:15 in eight tall glass cylinders. The cylinders were divided into two groups of four cylinders each. Each cylinder in one group received 50 mg N (as urea) per kg soil and one of the four nitrification treatments described above. The cylinders in the second group received 100 mg N (as urea) per kg soil plus one of the four treatments. The top of each cylinder was entirely covered by a large funnel. The tip of the funnel was connected to a polythene tube that dipped into a conical flask containing 100 ml of 0.02 (N) H_2SO_4. The cylinders were incubated 21 days and volatilized NH_3 was trapped in the acid of the conical flask. The residual acid content in the flask was titrated with standard alkali and the amount of NH_3-N in the acid solution was calculated from the titer values.

Results and Discussion

Pond soil properties are presented in Table 1. The soil was slightly acidic and deficient in

Table 1. General properties of the pond soil.

Property	*Value*
pH (1:2)	5.4
EC (dsm/cm)	1.25
Easily mineralizable nitrogen (mg/kg)	84
Mineralized nitrogen (mg/kg)	72.8
Organic carbon (%)	0.18
Available phosphorus (mg/kg)	10.5
Available potash (mg/kg)	86.5
Sand (%)	69
Silt (%)	19
Clay (%)	12
Texture	Sandy loam

available nitrogen. Available phosphorus and potassium were also low, indicating that the soil had low productivity and required fertilization.

Nitrification inhibitors tend to retard the rate of nitrification without affecting other biological activities in the system. Neem and karanj extracts, when mixed with nitrogenous fertilizers, reduce the rate of nitrification through the activity of their major components nimibidin and karanjanin, respectively (Mandal, 1984). Sodium azide (NaN_3) acts as a nitrification inhibitor by restricting the activity of nitrifying bacteria. The total mineralized NH_4^+ and NO_3^- in the soil in the fertilization treatments was considerably higher than in the unfertilized treatments (Table 2). Critical difference values show that the increments were statistically significant in most cases. Such behavior was attributed to the effects of the inhibitors in reducing denitrification of NO_3^--N in the semi-aerobic or anaerobic bottom sediments as discussed by Boyd (1995). Although not covered in this study, this behavior is likely to help reduce the leaching loss of highly soluble NO_3^--N from bottom soil.

The occurrence of easily mineralizable forms of N in the soil phase was similar to that of total mineralized nitrogen in the soil (Table 3). Easily mineralized nitrogen refers to organic nitrogen that can be transformed to a readily available state within a short period and includes the mineralized nitrogen forms (Subbiah and Asija, 1956). Since the nitrification inhibitors did not interfere with the mineralization of organic nitrogen to NH_4^+ but only restricted the nitrification process of NH_4^+ to NO_3^-, the inhibitors improved the amount of easily mineralizable N in all fertilizer treatments. The nitrification inhibitors helped maintain a higher concentration of NH_4^+-N. Generally, a good portion of NH_4^+-N remains adsorbed in the soil exchange complex and is unlikely to be lost from the pond environment except through volatilization.

The nitrification inhibitors increased the concentrations of water-soluble nitrogen in all the fertilization treatments (Table 4). A higher occurrence of water-soluble nitrogen is likely to benefit phytoplankton populations in the water, therefore, this beneficial effect of the inhibitors is particularly important.

The inhibitors increased the NH_4^+/NO_3^- ratios in all the fertilization treatments (Table 5). The relative concentrations of NO_3^- were higher in the water than in the soil, possibly due to the lower availability of oxygen in the bottom soils that restricted the transformation

Table 2. Effects of nitrification inhibitors on occurrence of mineralized NH_4^+-N and NO_3^--N in soil (mg/kg).

Treatment[1]	*Days of Incubation*					*Avg*
	15	*30*	*45*	*60*	*75*	
U_0	103.6	110.6	96.6	324.8	127.4	152.6[h]
U_0+N	135.8	191.8	140.0	394.8	154.0	203.28[e]
U_0+K	137.2	156.8	134.4	376.6	128.7	186.74[fg]
U_0+S	133.0	162.4	156.7	427.0	154.0	206.62[e]
U_{50}	119.0	156.8	131.6	333.2	139.1	175.93[g]
U_{50}+N	138.1	203.0	186.7	417.6	166.6	222.41[d]
U_{50}+K	165.2	183.4	187.6	432.6	168.0	227.36[d]
U_{50}+S	205.3	198.8	190.4	449.9	168.0	242.46[c]
U_{100}	135.8	182.9	160.5	352.8	147.5	195.9[ef]
U_{100}+N	166.6	218.4	267.3	457.3	172.2	256.35[b]
U_{100}+K	181.2	216.5	280.5	518.9	217.0	282.82[a]
U_{100}+S	224.9	324.8	164.3	477.4	245.5	287.37[a]
CD[2]	26.5	22.2	16.0	40.3	28.2	

Values followed by the same letter do not significantly differ at a 5% level of significance (Duncan's multiple range test).
[1] U_0 = no fertilization, U_{50} = 50 mg N (supplied as urea)/kg soil, U_{100} = 100 mg N (supplied as urea)/kg soil, N = neem (*Azadirachta indica*) extract, K = karanj (*Pongamia glabra*) extract, S = sodium azide (NaN_3).
[2] Critical difference, $p = 0.05$.

of nitrogen into NO_3^-. The oxygen in the water phase was higher, which may have counteracted some of the effects of the nitrification inhibitors. Phytoplankton consume NH_4^+ at a faster rate than NO_3^- and without expending much energy, another possible reason for the lower concentration of NH_4^+ ions in the water.

While denitrification of NO_3^- is less likely to occur in aerobic water, the comparatively lower occurrence of NH_4^+ in the water may reduce the magnitude of volatilization. To assess this possibility, a supplementary study was carried out. There were marginal increments in the amount of NO_3^- released from the system in the nitrification inhibitor treatments despite the very high doses of nitrogen, compared to the treatment with no inhibitor (Fig. 1). In addition to the lower NH_4^+ content in water, this could be attributed to the fact that standing water tends to dissolve a large part of the released NH_3 into NH_4OH, preventing its escape into the air.

The beneficial effects of using nitrification inhibitors together with nitrogenous fertilizers on the availability of nitrogen were reflected in the gross and net primary productivity (Table 6). The inhibitors resulted in considerable increases of gross and net primary productiv-

Table 3. Effects of nitrification inhibitors on easily mineralizable form of N in the soil (mg/kg).

Treatment[1]	*Days of Incubation*					*Avg*
	15	*30*	*45*	*60*	*75*	
U_0	128.8	156.8	100.8	182.0	105.0	134.68^{e}
U_0+N	301.0	275.8	141.4	263.3	168.0	229.9^{abcd}
U_0+K	166.2	189.0	131.6	240.8	158.2	177.16^{cde}
U_0+S	151.2	166.6	147.0	217.0	120.4	160.44^{de}
U_{50}	161.0	175.0	135.8	257.6	144.2	174.72^{cde}
U_{50}+N	323.0	313.6	150.6	274.4	212.8	254.88^{ab}
U_{50}+K	284.2	200.2	161.0	273.0	221.2	227.92^{abcd}
U_{50}+S	170.8	191.8	193.2	292.6	165.2	202.72^{bcde}
U_{100}	188.5	219.3	169.8	286.5	179.2	208.66^{bcd}
U_{100}+N	333.2	334.1	216.5	314.5	224.9	284.68^{a}
U_{100}+K	285.7	261.3	200.6	319.2	210.0	255.36^{ab}
U_{100}+S	210.0	254.8	196.9	328.5	206.2	239.28^{abc}
CD[2]	24.66	12.72	9.46	13.42	14.37	

Values followed by the same letter do not significantly differ at a 5% level of significance (Duncan's multiple range test).
[1] As in Table 2.
[2] Critical difference, $p = 0.05$.

ity, indicating that the resultant NH_4^+ was utilized in the food web. This finding is supported by the smaller NH_4^+/NO_3^- ratios in the water than in the soil. Since there was no distinct difference among the inhibitors with regard to primary productivity, any could be used to increase the efficiency of urea applied as a fertilizer in fishponds.

The study shows that the use of nitrification inhibitors may increase the efficiency of nitrogenous fertilizers in producing fish food organisms in fishponds. The benefit is achieved by reducing the transformation of the nitrogen in the fertilizer to nitrate and the subsequent loss of nitrogen through denitrification and other pathways.

Acknowledgement

The authors are thankful to the Indian Council of Agricultural Research for sponsoring the study.

Table 4. Effects of nitrification inhibitors on water-soluble nitrogen (NH_4^+-N and NO_3^--N; mg/l).

Treatment[1]	*Days of Incubation*					*Avg*
	15	*30*	*45*	*60*	*75*	
U_0	3.47	7.77	5.20	23.80	18.20	11.69[g]
U_0+N	6.77	8.40	9.52	28.14	24.92	15.55[ef]
U_0+K	4.81	8.96	6.26	27.72	26.60	14.87[f]
U_0+S	5.25	9.03	7.98	26.74	23.42	14.48[f]
U_{50}	4.14	9.33	8.17	25.30	21.70	13.73[f]
U_{50}+N	8.51	10.08	11.64	38.86	32.90	20.39[bc]
U_{50}+K	6.60	10.08	10.97	45.08	30.61	20.66[b]
U_{50}+S	7.05	13.09	8.95	33.97	29.96	18.60[cd]
U_{100}	6.02	10.31	10.48	27.81	30.18	16.96[de]
U_{100}+N	10.15	11.01	14.48	43.68	38.60	23.58[a]
U_{100}+K	7.95	12.18	12.35	51.61	33.60	23.34[a]
U_{100}+S	8.00	14.04	13.55	37.10	32.34	21.01[b]
CD[2]	1.639	2.006	2.762	13.501	5.962	

Values followed by the same letter do not significantly differ at a 5% level of significance (Duncan's multiple range test).

[1] As in Table 2.

[2] Critical difference, $p = 0.05$.

Table 5. Ratio between NH_4^+ and NO_3^- in soil and water.

Treatment[1]	*In soil*	*In water*
U_0	0.583	0.223
U_0+N	1.337	0.391
U_0+K	1.729	0.531
U_0+S	2.094	0.256
U_{50}	0.633	0.303
U_{50}+N	2.148	0.400
U_{50}+K	1.790	0.593
U_{50}+S	2.420	0.457
U_{100}	0.796	0.405
U_{100}+N	2.025	0.458
U_{100}+K	2.284	0.533
U_{100}+S	4.254	0.450

[1] As in Table 2.

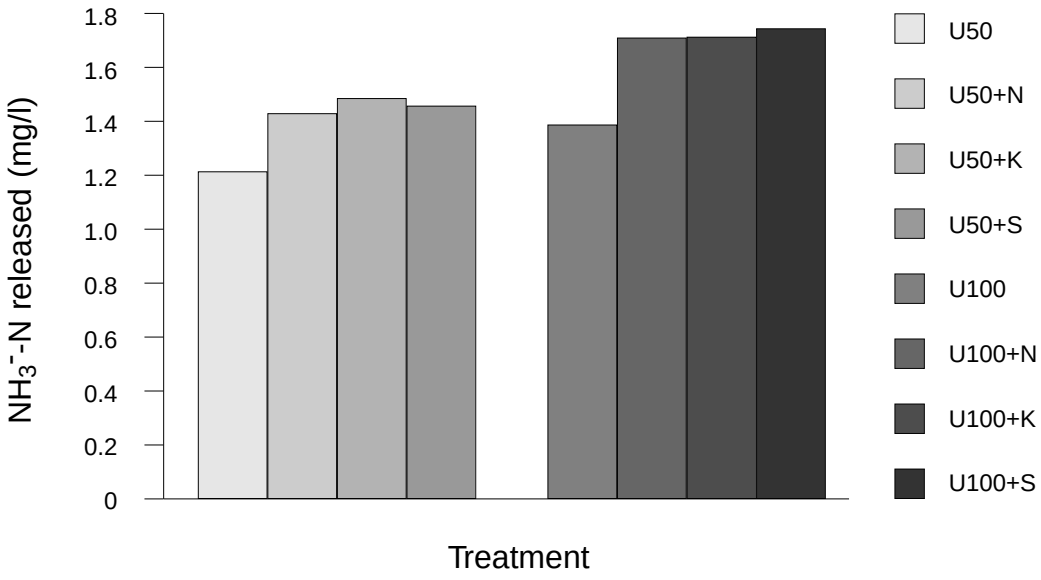

Fig. 1. Effect of nitrification inhibitors on release of NH_3-N.

Table 6. Primary productivity (mg C/m3/h) of water.

Treatment[1]	*Gross*	*Over no inhibitor (%)*	*Net*	*Over no inhibitor (%)*
U_0	77.078	-	33.326	-
U_0+N	102.076	32.43	54.160	62.52
U_0+K	131.178	70.19	87.490	162.53
U_0+S	120.828	56.76	66.656	100.01
U_{50}	109.710	-	59.022	-
U_{50}+N	158.522	44.49	70.828	20.00
U_{50}+K	170.732	55.62	89.570	51.76
U_{50}+S	179.160	63.30	85.410	44.71
U_{100}	152.760	-	82.630	-
U_{100}+N	238.160	55.90	110.208	33.38
U_{100}+K	248.598	62.74	148.604	79.84
U_{100}+S	237.494	55.47	145.132	75.64
CD[2]	81.4679	-	64.1803	-

[1] As in Table 2.
[2] Critical difference, $p = 0.05$.

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