Journal of Environmental Sciences Vol. 12, No. 1, pp. 125—128, 2000

ISSN 1001—0742
CN11—2725/X

Article ID: 1001-0742(2000)01-0125-04

Effects of allelochemicals on activity of nitrate reductase

MA Rui-xia

(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China)

Abstract: Study on the effects of allelochemicals such as *trans*-ferulic acid (*t*-FA), benzoic acid (BA) and *p*-hydroxybenzoic acid (*p*-HA), isolated from decomposed wheat straw on the activity of nitrate reductase at different concentrations of allelochemicals and different pH is described. *t*-FA (0.26, 2.58 and 5.15 mmol/L) and BA (4.09, 8.19 mmol/L) showed a certain inhibition to the activity of nitrate reductase. The highest inhibition rate was 18.40%, but BA (0.41 mmol/L) and *p*-HA (0.36, 1.81 and 3.62 mmol/L) showed stimulation, the more strong stimulation rate was 15.80%. At pH 6 condition, the activity of nitrate reductase was stronger inhibited than pH 7 and pH 8, but the mixture of 3 allelochemicals at pH 6 showed a stimulation. The mixture, however, at pH 7 and pH 8 showed some inhibition. It was found that there was a relationship between production of NO_2^- and transformation of NO_3^-.

Key words: allelochemicals; nitrate reductase; denitrification activity

CLC number: S131 **Document code:** A

Introduction

Plant straw as a important measure for increasing the fertility and improving the structure of soil, has been widely applied in agricultural practice. Many papers (Klmber, 1967; Hicks, 1989; Ramesh, 1990; Thome, 1990; Ma, 1993; 1996) indicated that a lot of so called allelochemicals would be produced during the decomposition of straw by soil microorganisms, and some of them may take part in nitrogen cycle in soil, and effected the utilization of nitrogen by plant.

Low utilization of nitrogen by plant (40%—50%) not only leads to a great economic loss, but also to serious environmental pollution. To study how to increase nitrogen utilization, and to elucidate the mechanisms and nitrogen loss way, have been a focus of agricultural investigation.

Denitrification (including chemical and biodenitrification) is a one of the important causes of nitrogen loss in soil. In this process the nitrate reductase plays an important role. Under anaerobic conditions, NO_3^- as an electron acceptor is reduced to NO_2^- by nitrate reductase, then NO_2^- is reduced to NO, N_2O and N_2 under the action of nitrite reductase and other enzymes (Kaplan, 1985; Rebertson, 1991). Although enzymes in soil have been determined and many papers dealt with denitrification, but there is only a few research works concerning the nitrate reductase. Adbelmagid H. M. has studied the activity of nitrate reductase in different kinds of soil (Abdelmagid, 1987). Jaap Van Rijn *et al*. have studied effects of volatile fatty acid on the activity of nitrate reductase (Jaap, 1996). However, how do the allelochemicals produced from the decomposition of plant straw in soil by the effect of nitrate reductase that is closely related to the denitrification has not been conducted. In order to increase the nitrogen utilization, it is of a great importance for understanding of loss process, mechanisms and ways of nitrogen loss. This project based on the chemical ecology is to study the effects of 3 main allelochemicals from the decomposition of wheat straw, such as *t*-FA, BA and *p*-HA at different concentration and pH on the activity of nitrate reductase, to discuss the promoting of the transformation of NO_3^- to NO_2^- by effect of allelochemicals on the activity of nitrate reductase in soil, as well as their relationship between NO_3^- and NO_2^-. Thus, it could present theoretical and practice bases for increasing N uptake by plant by means of regulatory eco-environmental condition and agricultural practices.

1 Materials and methods

The experiments were conducted in the laboratory. Test soil was collected from a farm of

Foundation item: National Natural Science Foundation of China (No. 39790100)

Pinggu, Beijing. The moisture content of the soil was 5%, and its pH was 7.15. The soil was sifted through 1 mm mesh and mixed with KNO_3 at a proportion of 98.9 μmol of NO_3^-/g dry soil. 1.05g of soil was transferred to test tube with allelochemicals added and NO_3^- of above proportion. A soil without allelochemicals was used as the control. Each treatment was in three replicates. Each test tube (1.8×20 cm) was degassed and flushed by N_2, sealed and then incubated at 30℃. The concentrations of nitrate and nitrite were measured by spectrophotometer after 24h incubation. In the experiments, pH values of samples were adjusted to 6, 7 and 8 with KOH. Denitrification activity was expressed by residues of nitrate and production of nitrite. Nitrates and nitrites were measured according to the method by Zheng Hongyuan (Zheng, 1982).

2 Results

Table 1 shows that the activity of nitrate reductase was inhibited by *t*-FA. Inhibition of *t*-FA at 2.58 mmol/L was stronger than 0.26 mmol/L and 5.15 mmol/L. The inhibition rate was 11.65%, and the formation of NO_2^- was 0.4 μmol/g dry soil less than the control.

Table 1 Influence of *t*-FA on nitrate reductase activity

Concentration, mmol/L	Residues of NO_3^-, μmol/g dry soil	Production of NO_2^-, μmol/g dry soil	Inhibition/Stimulation, −%/+%
Control	38.8	10.5	—
0.26	39.0	10.4	−0.52
2.58	43.3	10.1	−11.65
5.15	42.3	10.6	−9.02

The nitrate reductase activity was stimulated by *p*-HA (Table 2). The stimulation of *p*-HA was obvious at 1.81 mmol/L. The stimulation rate was 15.80%.

Table 2 Influence of *p*-HA on nitrate reductase activity

Concentration, mmol/L	Residues of NO_3N, μmol/g dry soil	Production of NO_2N, μmol/g dry soil	Inhibition/Stimulation −%/+%
Control	48.1	5.67	−
0.36	44.8	6.18	+6.86
1.81	40.5	6.42	+15.8
3.62	43.9	6.02	+8.73

The activity of nitrate reductase was inhibited by BA at 4.09 and 8.19 mmol/L (Table 3). The production of NO_2^- was decreased 5.0 μmol/g and 5.2 μmol/g dry soil compared with control, respectively. The treatment with 0.41 mmol/L of BA stimulated activity of nitrate reductase but the stimulation rate was lower than 0.36, 3.62 and 7.24 mmol/L of *p*-HA.

Table 3 Influence of BA on nitrate reductase activity

Concentration, mmol/L	Residues of NO_3^-, μmol/g dry soil	Production of NO_2^-, μmol/g dry soil	Inhibition/Stimulation −%/+%
Control	35.8	25.0	−
0.41	34.7	20.1	+3.07
4.09	42.4	20.1	−18.40
8.19	38.3	19.8	−6.98

Table 4 and 5 show the influences of *t*-FA and BA on the activity of nitrate reductase at different pH values. Less 6.6 μmol/g dry soil and 4.5 μmol/g dry soil of NO_3^- was reduced at pH 6, in the treatment of BA and FA (compared with control), respectively. Accumulation of NO_2^- was also lower by 4.9 μmol/g dry soil, in the treatment of BA than that of the control. The results showed that the inhibition is stronger under the condition of pH 6 than pH 8 and pH 7.

Table 4 Influence of *t*-FA (2.56 mmol/L) on nitrate reductase activity at different pH

pH	Treatment	Residues of NO_3^-, μmol/g dry soil	Production of NO_2^- μmol/g dry soil	Inhibition/Stimulation − %/+ %
6	Control	38.8	10.5	−
	t-FA	43.3	10.1	−11.600
7	Control	51.2	20.5	−
	t-FA	51.6	21.7	−0.781
8	Control	75.4	16.1	−
	t-FA	78.6	18.9	−4.24

Table 5 Influence of BA (4.09 mmol/L) on nitrate reductase activity at different pH

pH	Treatment	Residues of NO_3^-, μmol/g dry soil	Production of NO_2^-, μmol/g dry soil	Inhibition/Stimulation − %/+ %
6	Control	35.8	25.0	−
	BA	42.4	20.1	−18.4
7	Control	40.2	20.8	−
	BA	44.5	19.1	−10.7
8	Control	43.0	19.4	−
	BA	43.7	19.7	−1.63

The effects of pH of mixture of *t*-FA, BA and *p*-HA on nitrate reductase are summarized in Table 6. The residues of NO_3^- was reduced by 5.8 μmol/g dry soil, compared with control at pH 6. It indicated that the stimulation was apparent. These results demonstrated the stronger stimulation of *p*-HA than the inhibition of *t*-FA and BA under mixed at pH 6. The residues of NO_3^- was 4.4 μmol/g dry soil, more than control under pH 8. It indicated also inhibition.

Table 6 Influence of mixture of *t*-FA, BA and *p*-HA on nitrate reductase activity at different pH*

pH	Treatment	Residues of NO_3^-, μmol/g dry soil	Production of NO_2^-, μmol/g dry soil	Inhibition/Stimulation − %/+ %
6	Control	45.6	22.9	−
	Mixture	39.8	17.1	+12.7
7	Control	34.8	15.2	−
	Mixture	36.6	15.2	−5.17
8	Control	35.4	15.6	−
	Mixture	39.8	15.6	−12.4

* The concentration of *t*-FA, BA and *p*-HA was 2.58, 4.09, and 3.62 mmol/L, respectively in the mixture

3 Discussion

3.1 Effects of 3 allelochemicals at different concentrations on the activity of nitrate reductase

Different allelochemicals showed different effects on the activity of nitrate reductase. *t*-FA at 3 concentrations showed some inhibitions. BA at low concentrations (0.41 mmol/L) showed some stimulation, however, some inhibition to the activity of nitrate reductase at higher concentration (4.09 and 8.19 mmol/L). *p*-HA at all indicated concentrations showed its stimulation. The strong activity of nitrate reductase means a strong denitrification. Therefore, the results indicated that p-HA is not favorable for the conservation of N in agricultural field, and *t*-FA and BA play an active function for N conservation. These results provide a theorical basis for selecting nitrification inhibitors, *p*-HA stimulates N transformation, it provides a valuable data for N eutrophication in water pollution system.

3.2 Effects of allelochemicals at different pH on the activity of nitrate reductase

It is observed that BA at pH 6 and pH 7 showed the inhibition to the activity of nitrate

reductase. *t*-FA at pH 6 showed obviously inhibition, but at basic conditions (pH 7, pH 8) there is no inhibition at all. These results indicated that BA and *t*-FA, as denitrification inhibitor, could be used in neutral or light acidic soils, but not in basic soils. The mixture of three allelochemicals was studied at basic conditions, it displayed an obvious inhibition to the activity of nitrate reductase. However, it showed stimulation effect at pH 6. It could be understood that the stimulation caused by *p*-HA is stronger than the inhibition caused by *t*-FA and BA. Based on our results the mixture at basic environment could affectivity inhibit the activity of nitrate reductase, therefore, reduce the loss of nitrogen fertilizer.

Bremnet *et al*. (Bremnet, 1978) have found that the rate of denitrification increases with increasing pH and pH 7—8 was an optimum value. Jens *et al*. (Jens, 1994) have investigated the effects of different pH values on the denitrification and found the dynamic model of the nitrification at acidic and basic conditions to be different. Many researchers stated that the reductase at acidic conditions has been inhibited, therefore activity of denitrification was decreased. However Garcia has found the inhibition being stronger at basic conditions (Kypakoba, 1985). The effects of pH values in the soil on the denitrification process are considered to be more complex and different results were provided by researchers. Jens *et al*. (Jens, 1994) indicated that at the pH 5.5 NO_3^- at first transformed to NO_2^-, then to N_2O, and at pH 8.5 NO_3^- was directly transformed to N_2. In our experiments, NO_3^- could be transformed to NO_2^- at pH 8 and produced a large quantity of NO_2^-. This is quite different from Jens's results.

3.3 The relationship between NO_3^- transformation and NO_2^- production

In the experiments treated with *p*-HA at different concentrations the residues of NO_3^- was less than that in the control and NO_2^- produced was more than that in the control. In the experiments treated with *t*-FA and BA at pH 6, the residue of NO_3^- was more than that in the control, the amount transformed to NO_2^- was relatively less than that in the control. Thus, there was a relationship between NO_3^- transformation and NO_2^- production.

In the treatments of *t*-FA and BA at different concentration, the residues of NO_3^- is quite different, but the accumulation of NO_2^- is less different, because the competition between nitrate and nitrite for electrons is different (Jaap Van, 1996). In the experiments, *t*-FA and BA were used as the electron donor, *t*-FA and BA contain different electron flows and different pathways of transformation of electrons. In the incubation, their electron flow is changed, therefore different accumulation of nitrate and nitrite are influenced.

Acknowledgement: Thanks to Prof. Zhuang Yahui for his guidance.

References:

Abdelmagid H M, 1987. Soil Biol Biochem [J], 19(4):421—427.
Bremrer J M, Blackmer A M, 1978. Science [J], 199: 295—296.
Jaap Van Rijn, Yossi Tal, Yoram Barak, 1996. Appl and Environ Microbial [J], July: 2615—2620.
Jens K T, Torken G, Raymond P C, 1994. Applied and Environmental Microbiology [J], Feb: 536—541.
Hicks S K, Wendt C W, Gannanway J R, Baker R B, 1989. Crop Science [J], 29:1057—1061.
Kaplan W A, 1985. Adv Agric Microbiol [J], 3:186—206.
Klmber R W L, 1967. Aust J Agr Res [J], 18:361—374.
Kypakoba H T, 1985. Soil Advance [J], 13(5):8—13.
Ma Y Q, Zhang Y M, 1993. J of Chemical Ecology [J], 12(5):36—38.
Ma R X, Liu X F, Yuan G L, Sun S E, 1996. Acta Ecologica Sinica [J], 16(6):632—639.
Ramesh S H, Miller D A, 1990. Crop Sciences [J], 30:1255—1259.
Robertson L A, 1991. Microbial production and consumption of greenhouse gases: methane, nitrogen oxides and balomethanes [M]. Washindon D. C.: American Socity for Microbiology. 189—199.
Thome R L Z, Waller G R, Mcpherson J K *et al*., 1990. Bot Bull Academia Sinica [J], 31: 35—49.
Zheng H Y, Zhang D S, 1982. Study method of biochemistry in soil kinetic [M]. Beijing: Science Press. 180—182.

(Received for review May 12, 1998. Accepted September 16, 1998)