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Effect of Nitrification Inhibitors on Nitrogen Leaching and Enzyme Activities

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Abstract: Nitrification inhibitors (Thiourea and nitrification inhibitor "A") with urea fertilizer were used to enhance the nitrogen efficiency. Nitrification inhibitors and the amount of nitrogen were applied through urea fertilizer to rice seed in Column Lysimeters. The whole experiment was conducted with following treatments such as; urea application as a control at 2gN/10kg (200 kg N/ha), urea application 2gN/10kg (200 kg N/ha) added with 1.0%, 0.5%, 0.1%, 0.05% and 0.01% of thiourea (w/w) and urea application 2gN/10kg (200 kg N/ha) added with 1.0%, 0.5%, 0.1%, 0.05% and 0.01% of nitrification inhibitor "A" (w/w) alone and in combination form. Leachate samples were elicited from the Column Lysimeters and observed the NO_3 and NO_2 nitrogen after first, second, third and fourth weeks. The activity of NO_3 and NO_2 reductase also observed using UV-visible Spectrophotometer. It was found that all the treatments reduced nitrogen leaching and enzyme activities that involved in nitrification process. It was also observed that the 0.01% of thiourea, nitrification inhibitor "A" and thiourea with nitrification inhibitor "A" for decreased the NO_3 and NO_2 concentration in the leachating samples and also reduced the activities of NO_3 and NO_2 reductase in the soil after the fourth week.

Key words: Nitrification inhibitor · Urea · Thiourea · Enzyme · Reductase

INTRODUCTION

Nitrogen is an essential source for plant growth and reproduction. In Pakistan fertilizers are used to increases the crops yield [1]. Nitrogen is converted into available forms by microorganisms and some enzymes that present in soil [2]. Significant losses from some surface applied nitrogen sources can occur through the process of volatilization. In this process nitrogen is lost as ammonia (NH_3) gas. Nitrogen can be lost in this way from manure and fertilizer products containing urea. Ammonia is an intermediate form of nitrogen during the process in which urea is transformed to NH_4^+. Nitrogen sources are virtually eliminating volatilization losses. Loss of nitrogen from volatilization is greater when soil pH is higher than 7.3, air temperature is high, soil surface is moist and there is a lot of residue on the soil [3]. Water is a good solvent, it dissolves some toxic and hazard substances produced the water pollution problem. NO_3 and NO_2 concentration also increased in water with farming fertilizer, pesticide or poor sanitary activities. NO_3 nitrogen leaching from agricultural lands also threat against water. NO_3 and NO_2 contaminated the drinking water, nitrates undergo endogenous reduction and nitrosation of nitrites formed the carcinogens N-nitroso compounds. Nitrites led the infant's disease called methemoglobinemia [4, 5].

A potential method to reduce NO_3 nitrogen leaching into groundwater is the retardation of biological oxidation of ammonium nitrogen to NO_2 nitrogen [6]. In addition to nitrification, that produces nitrate. The concentrations of NO_3- also depend on the level of denitrification, the process that consumes NO_3- in the soil. NO_3 reductase (NaR) and NO_2 reductase (NiR) are the major enzymes involved in denitrification [7]. It has been reported that nitrification inhibitors can reduce nitrogen loss through denitrification [3]. Nitrification inhibitors are chemicals that slow down or delay the nitrification process and also used to enhance the nitrogen efficiency in soil [2]. The present research experiment was therefore mainly aimed to find out the effects of nitrification inhibitors with urea fertilizer on nitrate nitrogen enzyme activities involved in nitrogen cycling under the soil.

MATERIALS AND METHODS

Collection of Samples: The soil samples were collected from field "Department of Crop Physiology" University of Agriculture Faisalabad, Pakistan.

Fertilizer and Inhibitors Applications: Nitrification inhibitors and the amount of nitrogen were applied through urea fertilizer to rice seed in Column Lysimeters.

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The whole experiment was conducted with following treatments such as; urea application as a control 2gN/10kg (200 kg N/ha), urea application 2gN/10kg (200 kg N/ha) added with 1.0%, 0.5%, 0.1%, 0.05% and 0.01% of thiourea (w/w) and urea application 2gN/10kg (200 kg N/ha) added with 1.0%, 0.5%, 0.1%, 0.05% and 0.01% of unknown nitrification inhibitor "A" (w/w) alone and in combination form [8].

Nitrogen Leachating: Leachate samples were elicited from the Column Lysimeters the Nitrate nitrogen and Nitrite nitrogen were measured by the methods such as.

Analysis of Nitrate Nitrogen Leaching: Preparation of Reagent: 0.368 g Chromotropic acid was dissolved into 200 mL Conc. H_2SO_4 and kept the solution in a dark bottle. For analysis of NO_3 nitrogen took 3 mL leachate sample into conical flask, put the flask into cold water for a few minutes, add 1 mL chromotropic acid solution drop by drop directly into the leachating sample without mixing and again put into the cold water for 30 minutes. Mix the solution and add 6 mL Conc. H_2SO_4 on the flask wall without mixing. After adding the Conc. H_2SO_4 in the sample swirl the flask and leave to cool at room temperature for 45 minutes until the yellow color developed. The absorbance was measured at 430 nm by using UV-Spectrophtometer.

Analysis of Nitrite Nitrogen Leaching: Preparation of Reagents: The p-nitroaniline (0.2%) solution and diphenylamine (0.5%) solution were prepared in the ethanol. For analysis of NO_2 nitrogen took the 5 ml leachate sample add 2 mL of sulfuric acid solution, 1 mL of triton X-100 solution and 1 mL of p-nitroailine solution into a 10 mL volumetric flask. The solution was diluted up to 10 mL with distilled water. The solution was allowed to stand for 1 min followed by the addition of 1 mL of diphenylamine solution. Again the solution was allowed to stand for 10 min at the room temperature and absorbance was measured at 500 nm by using UV-Spectrophtometer [9].

Assay of Nitrie Reductase (Nir) and Nitrate Reductase (NaR) Activities: For the assay of NaR and NiR activities, 1 ml of 1% KNO_3 solution and 0.5% $NaNO_2$ solution were added to 1.0 g soil, respectively. The mixture were incubated at 30°C for 24 hours and the amount of reduced NO_3^--N and NO_2^--N were estimated to represent the activities of NaR and NiR respectively (Guan, 1986). And the reduced NO_3^--N was measured by the UV-Spectrophotometric method using chromotropic acid and NO_2^--N was also estimated by the Afkhami UV-Spectrophotometer nitrite determination method [9].

Statistical Analysis: Data was analyzed by applying the Analysis of Variance [10].

RESULTS AND DISCUSSION

Effect of Nitrification Inhibitors on the Growth: For observing the effect of nitrification inhibitors (Thiourea, Unknown nitrification inhibitor "A" and Thiourea with Unknown nitrification inhibitor "A") Rice seeds were grown in the lysimeters in triplicate form with various nitrification inhibitors treatments such as, 1.0, 0.5, 0.1, 0.05 and 0.01%. All these treatments were applied with the urea. Urea was used as nitrogen source for rice. After first, second, third and fourth week applying the treatments, rice growth were observed. The best rice growth was observed in the 0.01% of Thiourea with unknown nitrification inhibitor "A" treatment.

Effect of Thiourea on Nitrate Nitrogen (NO_3-N): The urea with the nitrification inhibitor Thiourea was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of nitrification inhibitor Thiourea on nitrate nitrogen was observed after first, second, third and fourth week and it decreased the NO_3-N concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 concentration but in the fourth week the treatment 0.01% of Thiourea showed the more efficiency for decreasing the NO_3-N concentration as shown in the Figure 1. When, urea was applied as nitrogen source to the rice. The nitrification inhibitor Thiourea inhibited the nitrate loss in the leaching samples and the concentration of NO_3 became low as by the inhibited the nitrification loss [11, 12].

Effect of Nitrification Inhibitor "A" on NO_3-N: The urea with the Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Nitrification inhibitor "A" on nitrate nitrogen was observed after first, second, third and fourth week and it decreased the NO_3-N concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 concentration but in the fourth week the treatment 0.01% of nitrification inhibitor "A" showed the more efficiency for decreasing the NO_3-N concentration as

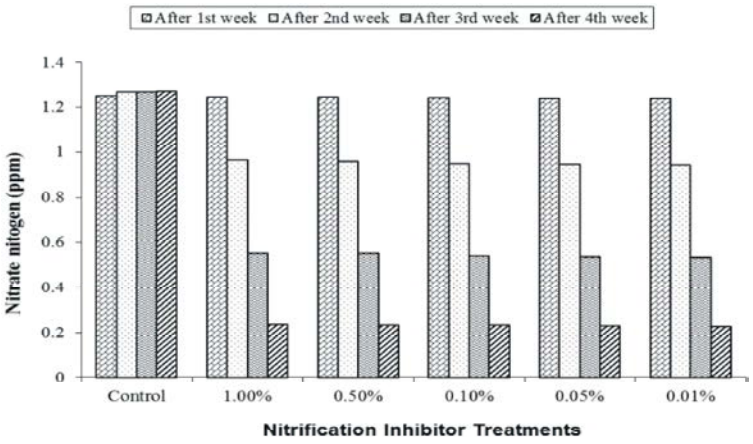

Fig. 1: Effect of Thiourea on Nitrate nitrogen

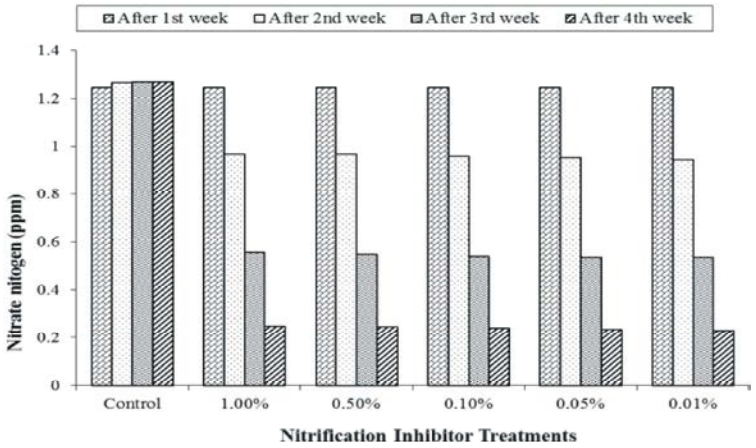

Fig. 2: Effect of Nitrification inhibitor "A"on Nitrate nitrogen

shown in the Figure 2. When, urea was applied as nitrogen source to the rice. The Nitrification inhibitor "A" inhibited the NO_3 loss in the leaching samples and the concentration of NO_3 became low as by the inhibited the nitrification loss [11, 13].

Effect of Thiourea with Nitrification Inhibitor "A" on NO_3-N: The urea, Thiourea with Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Thiourea with Nitrification inhibitor "A" on NO_3-N was observed after first, second, third and fourth week and it decreased the NO_3-N concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 concentration but in the fourth week the treatment 0.01% of Thiourea with Nitrification inhibitor "A" showed the more efficiency for decreasing the NO_3-N concentration as shown in the Figure 3. When, urea was applied as nitrogen source to the rice. The Thiourea with Nitrification inhibitor "A" inhibited the NO_3 loss in the leaching samples and the concentration of NO_3 became low as by the inhibited the nitrification loss [12].

Effect of Thiourea on Nitrite Nitrogen (NO_2-N): The urea with the nitrification inhibitor Thiourea was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of nitrification inhibitor Thiourea on NO_2-N nitrite nitrogen was observed after first, second, third and fourth week and it decreased the nitrite nitrogen concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the nitrate concentration but in the fourth week the treatment 0.01% of Thiourea showed the more efficiency for decreasing the NO_2-N concentration as shown in the Figure 4. When, urea was applied as nitrogen source to the rice. The nitrification inhibitor Thiourea inhibited the NO_2 loss in the leaching samples and the concentration of NO_2 became low as by the inhibited the nitrification loss [11, 12].

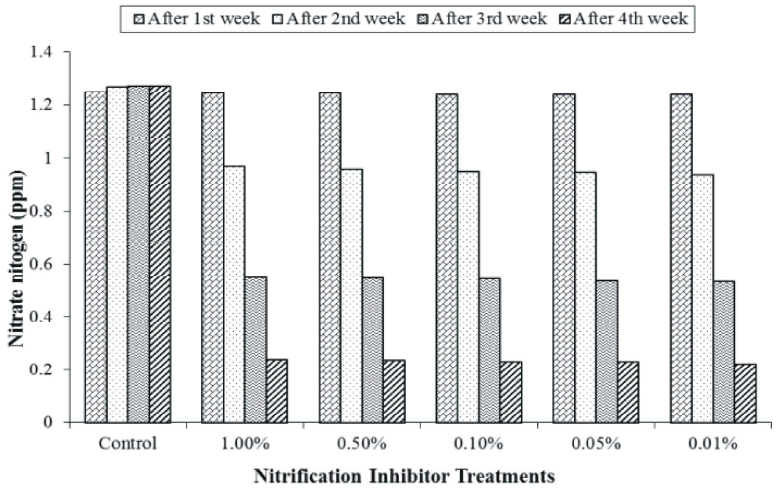

Fig. 3: Effect of Thiourea with Nitrfication inhibitor "A" on Nitrate nitrogen

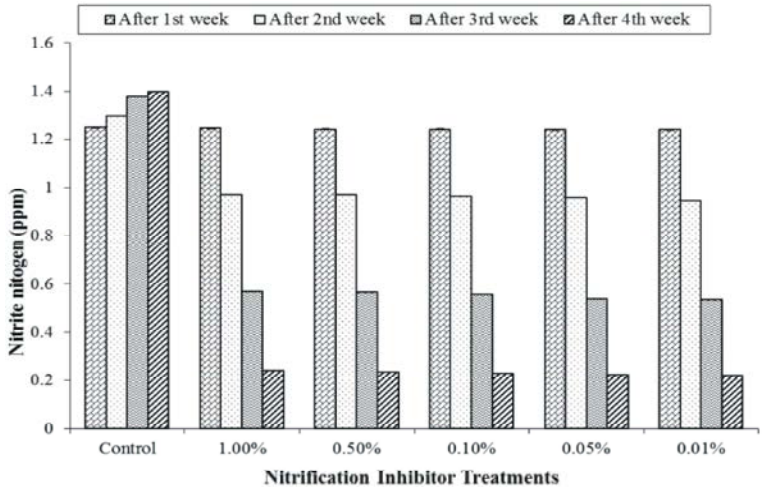

Fig. 4: Effect of Thiourea on Nitrite nitrogen

Effect of Nitrification Inhibitor "A" on Nitrite Nitrogen (NO_2-N): The urea with the Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Nitrification inhibitor "A" on NO_2-N was observed after first, second, third and fourth week and it decreased the nitrite nitrogen concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_2 concentration but in the fourth week the treatment 0.01% of Nitrification inhibitor "A" showed the more efficiency for decreasing the nitrite nitrogen concentration as shown in the Figure 5. When, urea was applied as nitrogen source to the rice. The Nitrification inhibitor "A" inhibited the NO_2 loss in the leaching samples and the concentration of NO_2 became low as by the inhibited the nitrification loss [12, 13].

Effect of Thiourea with Nitrification Inhibitor "A" on Nitrite Nitrogen (NO_2-N): The urea, Thiourea with Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Thiourea with Nitrification inhibitor "A" on NO_2-N was observed after first, second, third and fourth week and it decreased the NO_2-N concentration. There was highly significant difference between the varying treatments and week. All the treatments decreased the nitrate concentration but in the fourth week the treatment 0.01% of Thiourea with Nitrification inhibitor "A" showed the more efficiency for decreasing the NO_2-N concentration as shown in the Figure 6. When, urea was applied as nitrogen source to the rice. The Thiourea with Nitrification inhibitor "A" inhibited the NO_2 loss in the leaching samples and the concentration of nitrite became low as by the inhibited the nitrification loss [11, 12].

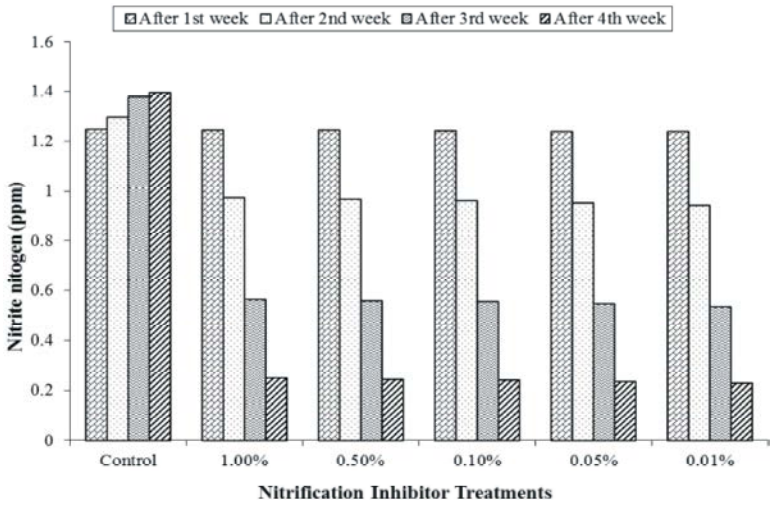

Fig. 5: Effect of Nitrification inhibitor "A" on Nitrite nitrogen

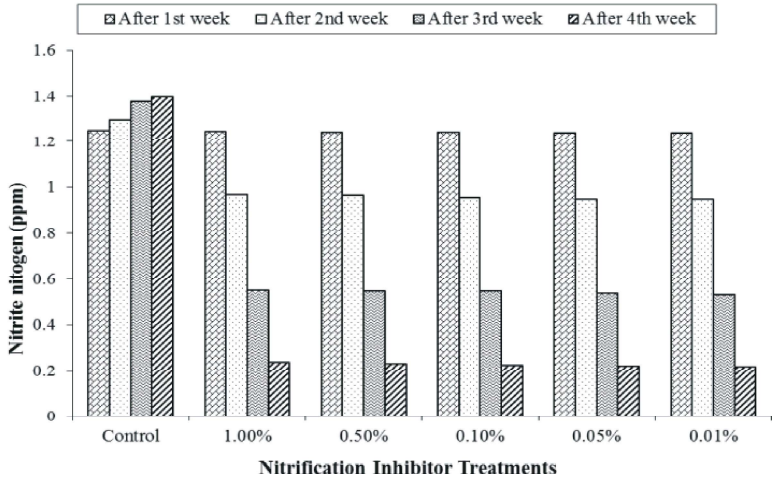

Fig. 6: Effect of Thiourea with Nitrification inhibitor "A" on Nitrite nitrogen

Effect of Thiourea on Nitrate Reductase (NaR): The urea with the nitrification inhibitor Thiourea was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of nitrification inhibitor Thiourea on NO_3 reductase was observed after first, second, third and fourth week and it decreased the NO_3 reductase activity. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 reductase activity but in the fourth week the treatment 0.01% of Thiourea showed the more efficiency for decreasing the NO_3 reductase activity as shown in the Figure 7. When, urea was applied as nitrogen source to the rice. The nitrification inhibitor Thiourea inhibited the NO_3 reductase that involved in the nitrification process [14, 15].

Effect of Nitrification Inhibitor "A" on Nitrate Reductase (NaR): The urea with the Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Nitrification inhibitor "A" on NO_3 reductase was observed after first, second, third and fourth week and it decreased the NO_3 reductase activity. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 reductase activity but in the fourth week the treatment 0.01% of Nitrification inhibitor "A" showed the more efficiency for decreasing the NO_3 reductase activity as shown in the Figure 8. When, urea was applied as nitrogen source to the rice. The Nitrification inhibitor "A" inhibited the NO_3 reductase that involved in the nitrification process [12, 13, 15].

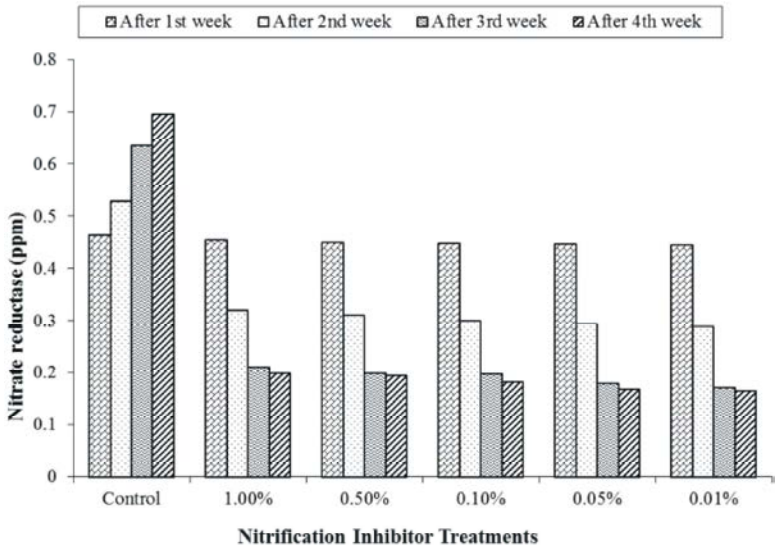

Fig. 7: Effect of Thiourea on Nitrate reductase (NaR)

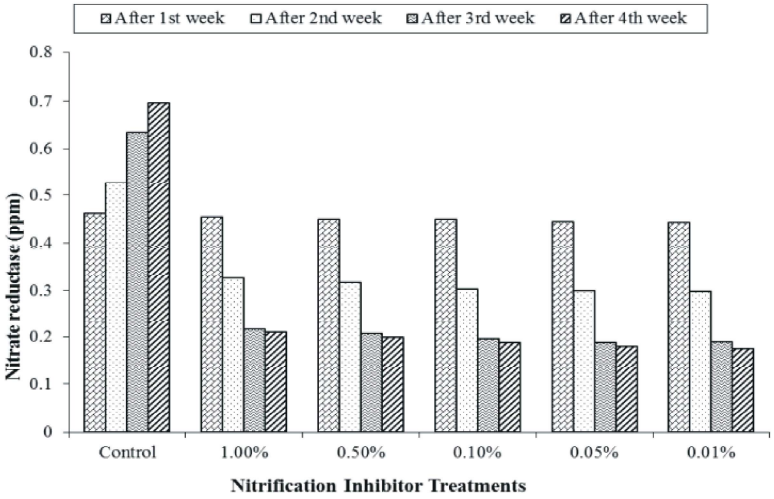

Fig. 8: Effect of Nitrification inhibitor "A" on Nitrate reductase (NaR)

Effect of Thiourea with Nitrification Inhibitor "A" on Nitrate Reductase (NaR): The urea, Thiourea with Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Thiourea with Nitrification inhibitor "A" on NO_3 reductase was observed after first, second, third and fourth week and it decreased the NO_3 reductase activity. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_3 reductase activity but in the fourth week the treatment 0.01% of Thiourea with Nitrification inhibitor "A" showed the more efficiency for decreasing the NO_3 reductase activity as shown in the Figure 9. When, urea was applied as nitrogen source to the rice. The Thiourea with Nitrification inhibitor "A" inhibited the NO_3 reductase that involved in the nitrification process [11, 13, 15].

Effect of Thiourea on Nitrite Reductase (NiR): The urea with the nitrification inhibitor Thiourea was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of nitrification inhibitor Thiourea on NO_2 reductase was observed after first, second, third and fourth week and it decreased the nitrite reductase activity. There was highly significant difference between the varying treatments and week. All the treatments decreased the NO_2 reductase activity but in the fourth week the treatment 0.01% of Thiourea showed the more

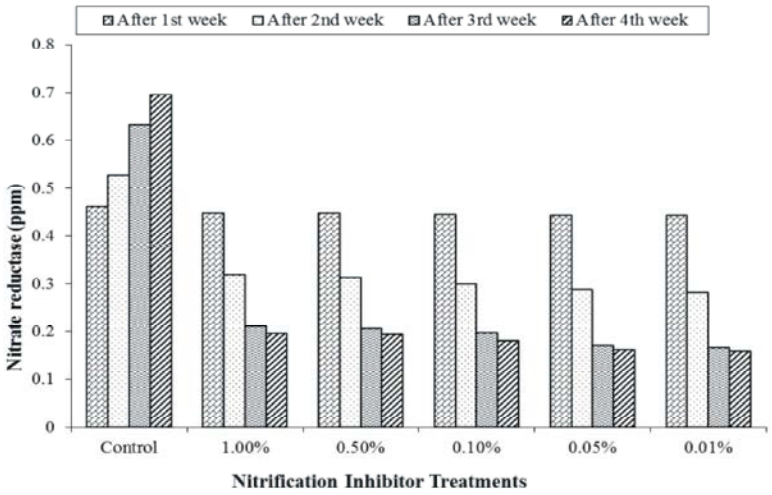

Fig. 9: Effect of Thiourea with Nitrification inhibitor "A" on Nitrate reductase (NaR)

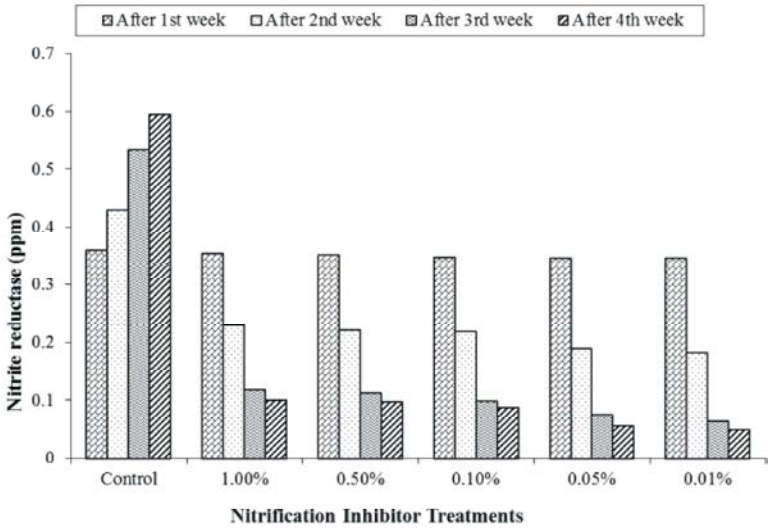

Fig. 10: Effect of Thiourea on Nitrite reductase (NiR)

efficiency for decreasing the NO_2 reductase activity as shown in the Figure 10. When, urea was applied as nitrogen source to the rice. The nitrification inhibitor Thiourea inhibited the NO_2 reductase that involved in the nitrification process [11, 14, 15].

Effect of Nitrification Inhibitor "A" on Nitrite Reductase (NiR): The urea with the Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Nitrification inhibitor "A" on NO_2 reductase was observed after first, second, third and fourth week and it decreased the NO_2 reductase activity. There was highly significant difference between the varying treatments and week. All the treatments decreased the nitrite reductase activity but in the fourth week the treatment 0.01% of Nitrification inhibitor "A" showed the more efficiency for decreasing the NO_2 reductase activity as shown in the Figure 11. When, urea was applied as nitrogen source to the rice. The Nitrification inhibitor "A" inhibited the NO_2 reductase that involved in the nitrification process [11-13, 15].

Effect of Thiourea with Nitrification Inhibitor "A" on Nitrite Reductase (NiR): The urea, Thiourea with Nitrification inhibitor "A" was applied with the treatments such as 1.0, 0.5, 0.1, 0.05 and 0.01% in triplicate form. The effect of Thiourea with Nitrification inhibitor "A" on nitrite reductase was observed after first, second, third and fourth week and it decreased the NO_2 reductase activity. There was highly significant difference between

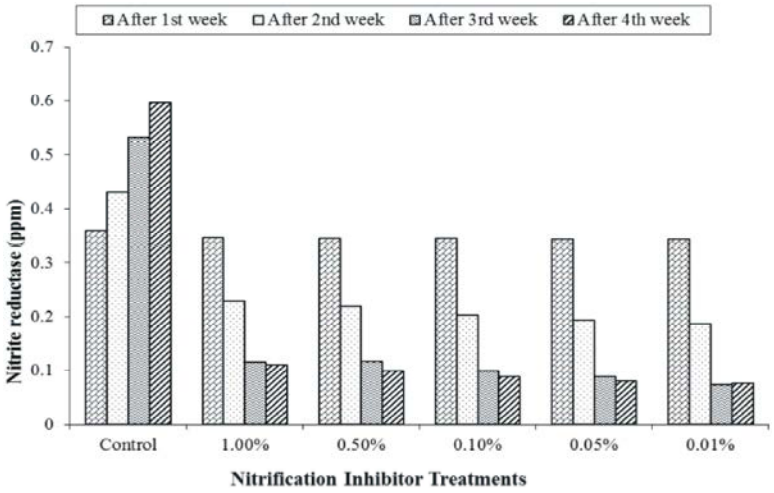

Fig. 11: Effect of Nitrification inhibitor "A" on Nitrite reductase (NiR)

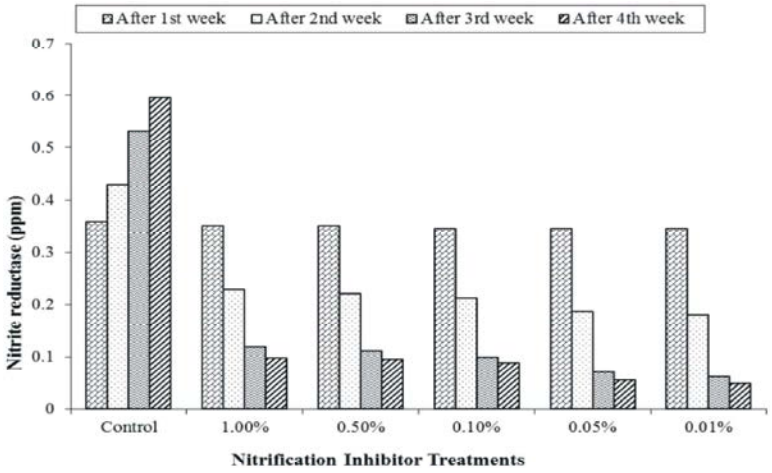

Fig. 12: Effect of Thiourea with Nitrification inhibitor "A" on Nitrite reductase (NiR)

the varying treatments and week. All the treatments decreased the NO_2 reductase activity but in the fourth week the treatment 0.01% of Thiourea with Nitrification inhibitor “A” showed the more efficiency for decreasing the NO_2 reductase activity as shown in the Figure 12. When, urea was applied as nitrogen source to the rice. The Thiourea with Nitrification inhibitor “A” inhibited the NO_2 reductase that involved in the nitrification process [11, 12].

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