# The Effect of Neem (*Azadirachta indica*) Coated Urea Fertilizer against NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> Concentration in Clay Loam Soil Texture

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ABSTRACT. The study aims to determine the level  $NH_4^+$  and  $NO_3^-$  in the soil with clay loam texture with addition neem coated urea fertilizer. This study was conducted in Experimental Farm of Biology Faculty of Mathematic and Natural Science Makassar State University and Laboratory of Assessment Institute for Agriculture Technology in Maros. This research was an experimental study using a Completely Randomized Design (CDR) with five treatments are control, urea, neem (2,5%) coated urea, neem (5%) coated urea and DCD (5%) coated urea with three replicates. Analysis of  $NH_4^+$  and  $NO_3^-$  levels in this study using colourimetric method. Results obtained by the levels of  $NH_4^+$  and  $NO_3^-$  produced after applied of nitrification inhibitor materials coated urea fertilizer as indicated by concentration of  $NH_4^+$  and  $NO_3^-$  produced in soil. Neem (5%) coated urea as a nitrification inhibitor showed the best effect in increasing levels of  $NH_4^+$  in the soil, while DCD (5%) coated urea were the best inhibitor in reducing levels of  $NO_3^-$  in the soil.

Key words: Ammonium, Nitrate, Neem Coated Urea Fertilizer, Clay Loam Soil texture.

## **INTRODUCTION**

Soil is very important for all life on earth as the ground supporting the life of plants by providing nutrients and water as well as the support of the root. Soil structure, hollow-cavity also be a good place for the roots to breathe and grow. Land is also a habitat for various living microorganisms. For most of the animals, land becomes place to live and move and is very useful for human life. Soil fertility is the ability of soil to be able to provide nutrients to the needs of development and growth and crop production. Nitrogen is the main nutrient for plant growth, which in general is very necessary for the formation and growth of vegetative parts of the plant such as roots, stems and leaves (Sutedjo, 2008). The availability of nitrogen in the soil is relatively small, but the amount required by the plant is very large. Therefore it is necessary for fertilization to increase the amount of nutrients in the soil. One fertilizer that is often used by farmers for crop fertility is urea. Its popularity is mostly due to the high levels of nitrogen and the price is cheap (Indranada, 1985). Nitrogen in fertilizers is generally in the form of N-ammonium (NH4 +), which is then rapidly converted to nitrate in the soil by nitrification. Therefore, excessive fertilizer will increase the nitrate content in the soil (Yuningsih, 2007). Nitrate (NO3) quickly leached into the water and if the environment allows, these compounds continue to be converted into gas N2, N2O and NO through denitrification causing the majority of nitrogen in fertilizers can not be used by plants (Poerwowidodo, 1992). The leaching of nitrate in large quantities will pollute the waters that will cause eutrophication of aquatic weeds (Anggrahini, 2009). Seeing from the negative impacts caused by the fertilization, there are currently two nitrification inhibiting the rate synthetic materials widely known because it has been tested and commercially traded that nitrapyrin

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and dicyandiamide (DCD). Aside from the synthetic inhibiting the rate of nitrification, nitrification inhibitors unisex natural material that is extracted from a particular part of the plant neem (*Azadirachta indica*). Effects of plant neem such as inhibitors of nitrification have been tested and have real impact on some of the indicators to be achieved include  $N_2O$  diminishing population and oxidizing bacteria nitrates and oxidizing bacteria ammonium increased which helps in the enrichment of the soil (Jumadi et al, 2008).

Dicyandiamide is an example of the nitrification inhibitor compounds that can inhibit the first stage process of nitrification is the oxidation of  $NH_4^+$  to  $NO_2$  and inactivate enzymes produced by nitrification bacteria that AMO (ammonium monooxygenase). In addition DCD also contain N which serves as a nitrogen fertilizer. While neem (*Azadirachta indica*) is a plant that acts as an organic fertilizer that contains nutrients that are essential to the plant. Neem seeds contain a chemical that is commonly referred to as triterpenes, which can inhibit activity of bacteria that play a role in the process of nitrification. If the dregs of neem (*Azadirachta indica*) and dyciandiamide (DCD) mixed with urea in a coated way the use of urea will be more efficient.

Based on the above, we conducted this study to examine the effect of using urea coated neem (*Azadirachta indica*) on levels of  $NH_4^+$  and  $NO_3$  on maize with clay loam soils. The barrier materials used in this research that the seed extract of neem (*Azadirachta indica*) and dicyandiamide (DCD). In this research granular urea coated process using oil seed extraction and neem (*Azadirachta indica*) and dicyandiamide (DCD) is considered as an effective way to inhibit nitrification and easier to apply in the field.

#### **RESEARCH METHODS**

The research is experiment by measuring the levels of ammonium  $(NH_4^+)$  and nitrate  $(NO_3)$  on clay loam soils. As for the equipment and materials used are: spectrophotometers, corresponding cuvettes, vortex, shakers, centrifuges, stopwatch, test tubes, bottles shake plastic, beakers, measuring cups, tube rack, pipette volumes of 1, 2 and 5 ml, analytical balance, Erlenmeyer and flask, clay loam soil textured, dregs neem, dicyandiamide (DCD), oils of neem, sucrose, an alkali solution, urea granule, GSP (granules Sulfur Phosphate), KCL (potassium chloride), aquabidest, solution Sangga Tartrate, NaOH, K1 Na -Tartrat, a solution of Na-phenate, Phenol, Sodium Hypochlorite (NaOCl) 5%, Brucine 2%, sulfuric acid (H2SO4), Ammonium Sulfate (NH4) 2SO4, KNO3, tissue and plastic.

**Research Procedure** 

1. Preparation Material of Nitrification inhibitors

In this study, the raw material is mixed with neem and DCD granular urea by way coated process. Neem dose used was 2.5% and 5%, while the DCD dosage is 5%. Neem dose of 2.5% means that in 100 grams of urea coated by 2.5 grams neem neem as well as a dose of 5% and 5% DCD. Coated process granular urea by neem appropriate dose of 2.5% and 5% urea granule sprayed with neem oil is combined with water at a dose of 1% using lye soap (1 ml / 500 ml H2O) and then sprinkled with neem seed extract in the form neem powder to a final concentration of 2.5% and 5%, after penyelaputan uneven, granular urea fertilizer coated neem dried using sunlight. The process of urea granules coated by DCD 5% urea granule sprayed with sucrose combined with water at a dose of 1% (99 ml H2O + 0.1 gram of sucrose) and then sprinkled with DCD 5%, after coated evenly, urea ganul coated DCD dried using sunlight.

2. Treatment

In this study use of urea, urea coated neem 2.5%, urea coated neem 5%, urea coated DCD 5%, KCl and GSP. Fertilization in maize done 2 times. Fertilization is first carried out a week after planting maize. Fertilizer coated nitrification inhibitors embedded around the corn plant with a distance of 5 cm. Second fertilization is done after the corn crop was four weeks, only urea fertilizer, KCL (potassium chloride) and GSP (sulfur granules phosphate) by maize.

3. Sampling

Soil sampling was conducted 4 times. The first soil sampling performed on the  $10^{\text{th}}$  days after planting the corn. The second soil sampling performed on the  $25^{\text{th}}$  days after planting the corn. Sampling is done on the third  $40^{\text{th}}$  days after planting corn and sampling last performed on  $55^{\text{th}}$  days after planting maize. Soil sampling carried out evenly by taking soil from a depth of 0-10 cm from ground level (top soil), it is done because the soil used is part of top soil has good aeration and a part where the plants grow as well as many contain ingredients organic. Clay loam soil texture is taken from the ground maize (*Zea mays*) in the experimental garden Green House Biological Science UNM Makassar.

4. Preparation of soil extract

A total of 10 grams of soil sample was dissolved in 20 ml aquabidest, then dishake for 30 minutes at a speed of 200 rpm, then dicentrifuge for 35 minutes to obtain a soil extract.

5. Analysis of the levels of  $NH_4^+$ 

Pipette into test tubes each 2 ml of extract soil and add a row prop tartrate solution, Na-phenate and natriun hypochlorite (NaOCl) 5% respectively of 4 ml then homogenized using a vortex. After that, waited for 30 minutes and then measured with a spectrophotometer at a wavelength of 636 nm to obtain the absorbance value.

6. Analysis of the levels of NO<sub>3</sub><sup>-</sup>

Pipette each 2.5 ml of extract ground into a test tube. Add 0.25 ml of brucine afterwards homogenized using a vortex and then wait for 10 minutes. After 10 minutes add sulfuric acid (H2SO4) by 2.5 ml concentrated and homogenkan using a vortex and wait for 30 minutes. After 30 minutes, the solution is measured with a spectrophotometer at a wavelength of 432 nm to obtain the absorbance value.

7. Analysis of Data

Data obtained from observations were analyzed using analysis of variance (F test) at the level of  $\alpha$ : 0.01. If these tests are real differences then the test continued with LSD (Least Significant Difference).

## **RESULTS AND DISCUSSION**

## A. Results

Measurement of levels of ammonium  $(NH_4^+)$  and nitrate  $(NO_3^-)$  on clay loam soil textured after planting and fertilizer urea coated nitrification inhibitors showed different results. Data can be seen as follows:

Table 1. Average levels of ammonium (mg N g-1 soil) on clay loam soils in the old days after treatment with urea application coated nitrification inhibitors.

_	Average levels of ammonium (mg N g-1 soil) days after planting				
Treatment					
	10	25	40	55	
К	3,26 <sup>c</sup>	2,16 <sup>c</sup>	12,70 <sup>c</sup>	3,25 <sup>b</sup>	
U	23,66 <sup>b</sup>	12,25 <sup>b</sup>	19,57 <sup>b</sup>	33,08 <sup>a</sup>	
А	55,97 <sup>a</sup>	17,03 <sup>b</sup>	$20,90^{b}$	4,92 <sup>b</sup>	
М	22,46 <sup>b</sup>	39,30 <sup>a</sup>	39,05 <sup>a</sup>	$2,48^{b}$	
D	$44.76^{a}$	$21,38^{ab}$	20,31 <sup>b</sup>	$2.27^{b}$	

Note: Figures are given the same letters in the same column means are not significantly different at the level of  $\alpha$ : 0.01



Figure 1. Average levels of  $NH_4^+$  (N g g-1 soil) produced on clay loam soil textured after treatment with urea fertilizer application coated nitrification inhibitors.

 Table 2. Average levels of nitrate (mg N g-1 soil) on clay loam soils in the old days after treatment with urea application coated nitrification inhibitors.

Treatment	Average levels of nitrate (mg N g-1 soil) days after planting				
	10	25	40	55	
K	7,53 <sup>b</sup>	5,60 <sup>b</sup>	$16,26^{ab}$	6,58 <sup>b</sup>	
U	14,69 <sup>a</sup>	$7,00^{a}$	$17,48^{a}$	$18,76^{a}$	
А	16,43 <sup>a</sup>	6,69 <sup>ab</sup>	12,57 <sup>c</sup>	$4,13^{bc}$	
Μ	15,63 <sup>a</sup>	7,31 <sup>a</sup>	12,54 <sup>c</sup>	1,24 <sup>c</sup>	
D	$14,75^{a}$	4,04 <sup>b</sup>	15,12 <sup>b</sup>	$0,63^{c}$	

Note: Figures are given the same letters in the same column means are not significantly different at the level of  $\alpha$ : 0.01



Figure 2. Average levels of NO<sub>3</sub><sup>-</sup> (mg N g-1 soil) produced on clay loam soil textured after treatment with urea application coated nitrification inhibitors.

## **B. DISCUSSION**

1. Levels of ammonium  $(NH_4^+)$ 

Based on analysis of the levels of ammonium in the texture of clay on  $10^{\text{th}}$ ,  $25^{\text{th}}$ ,  $40^{\text{th}}$  and  $55^{\text{th}}$  days after planting the obtained results in Figure 1 which shows that at  $10^{\text{th}}$  days levels of ammonium higher in all treatments were given urea except control with BNT value = 18.42. It caused no additional nitrogen derived from urea or from neem and DCD. Sources of nitrogen contained in the control plot is purely derived from the decomposition of organic matter in the soil, so that the lower levels of ammonium.

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High levels of ammonium in the treatment of urea but not as high as levels in the treatment of urea coated neem 2.5% and urea coated DCD 5%. Urea given start experiencing amonification where according to Hanafi (2010) to decompose urea into ammonium by the enzyme urease to accelerate the formation reaction of ammonium. So the availability of urea ammonium in the plot too high. According to Hakim et al (1986) of nitrogen fertilizer added to the soil will concentrate very high compared to land that is not in fertilizers. Ammonium levels are also high in urea treatment coated neem 5% but not as high as with the treatment of urea, urea coated neem 2.5% and urea coated DCD 5%. It caused the soil microorganisms have not been effective in decomposing organic matter in manure and neem neem saw a sizeable concentration of 5%. A large amount of nitrogen in the soil is to be in organic form. Thus the decomposition of nitrogen is the main source of soil nitrogen. Decomposition is a chemical process that produces nitrogen in the ammonium form and oxidised again into nitrite. Urea ammonium levels in the treatment of membranous neem 2.5% and urea treatment coated DCD 5% is very high. This is due to the addition of urea coated neem and DCD who have power in inhibiting nitrification rate, so the availability of ammonium  $(NH_4^+)$  in the high ground. Besides material containing neem and DCD also nitrogen so high ammonification process likely to take place primarily in the addition of neem, but the amount of nitrogen have already converted or included in the dosage of fertilizer.

Besides the presence of nitrification inhibitors, soil texture also affects the availability of nutrients in the soil. Where in this study used textured clay soil sand fraction with a proportion of 24%, the dust fraction of 47% and 29% clay fraction. The fraction of dust that dominate in clay loam soil texture generate power strong enough grasp of the water. This leads to relatively good soil drainage.

Observations on  $25^{\text{th}}$  days in Figure 1 shows ammonium levels decreased in all treatments except the treatment of urea coated neem 5% to the value of the BNT = 16.81. This is due in part ammonium been absorbed by corn plants and partly undergo nitrification and denitrification. Purwanto (2009) nitrification is the oxidation of ammonium with oxygen into nitrite followed by the oxidation of nitrite to nitrate, in other words nitrification is the process by which ammonium is converted to nitrite (NO<sub>2</sub>) and then nitrate (NO<sub>3</sub>). This process occurs naturally in the environment and is done by a group of bacteria Nitrosomonas and Nitrobacteria. Nitrification is an important step in the nitrogen cycle in the soil in which ammonium (NH<sub>4</sub><sup>+</sup>) an initial substrate for nitrification. While the treatment of urea ammonium levels coated neem 5% increase, this is caused by soil microorganisms has been active in decomposing organic material of neem 5% and experienced amonification in the soil that are available in large quantities.

Observations on  $40^{\text{th}}$  days in Figure 1 shows ammonium levels decreased slightly in the treatment of membranous DCD 5% urea and urea coated neem 5% whereas in control, urea and urea coated neem 2.5% experienced a slight increase in the value of BNT = 2,86. Chances are this is due to happend amonification namely ammonium formation process of the organic materials contained in the soil. This is supported by the N content in clay loam soils are generally higher. While the content of urea ammonium decreased in urea coated DCD 5% and neem 5% as ammonium contained in the soil has already been taken or absorbed by corn plants.

Observations on  $55^{\text{th}}$  days in Figure 1 shows ammonium levels decreased in all treatments except treatment of urea to the value of the BNT = 14.95. Urea ammonium levels increased in the plot caused by the second urea fertilizer as much as 4.82 grams. According Pitojo (1995) amonifikasi speed urea is affected by temperature, humidity, pH and soil types. Amonification generally takes place more rapidly in the argillaceous soil than sandy soil. Similarly, the high-temperature ground amonification faster than ground low temperature and wet than dry. So the availability of high-ammonium on the plot. Ammonium levels in the treatment of urea decreased coated neem 2.5%, neem 5% and DCD 5% due to ammonium contained in the soil is used up or absorbed by the corn plant or lost in the form of nitrate leached.

2. Levels of Nitrate  $(NO_3)$ 

Based on analysis of high nitrate levels in the texture of clay on  $10^{\text{th}}$ ,  $25^{\text{th}}$ ,  $40^{\text{th}}$  and  $55^{\text{th}}$  days after planting the obtained results in Figure 2 which shows that on the 10th day of nitrate levels tend to be low in all treatments, especially in the control value BNT = 3.76. This is because the treatment is not given urea coated nitrification inhibitors that nitrate levels are very low. Then followed by treatment of membranous neem 5% urea, urea treatment urea coated DCD 5% and 2.5% neem. Low levels of nitrate in the treatment due to the provision of urea fertilizer coated nitrification inhibitors that neem seed extract and DCD which can affect the rate of nitrification is indicated by high levels and low levels of ammonium nitrate. This is due to the chemical content of materials used nitrification inhibitors able to inhibit nitrification in the soil. According to Sharma and Prasad (1996), the chemicals contained in the seeds of neem able to inhibit microbial activity is responsible for nitrification (nitrifying), precisely the inhibition process of changing a compound of ammonium to nitrite thus enabling availability will be N-ammonium in the soil can be absorbed by plants,

Observations on  $25^{th}$  days in Figure 2 shows the nitrate levels experienced a sharp decline in all treatments with LSD value = 1.22. Chances are this is due to the rainfall intensity is very high at the time of

the study so that the nitrate in the soil experienced a run-off or leaching and their uptake by corn plant. Nitrate  $(NO_3^-)$  is unstable, so easily carried away by the water. Nitrate is an ion that is easy to move in the soil. This is due once it is easily soluble and adsorbed by soil colloids. Unavailability of N from the soil can be through a process of washing / leached (leaching)  $NO_3^-$ ,  $NO_3^-$  denitrification into  $N_2$ ,  $NH_4^+$  volatilization into  $NH_3^-$  or consumed by soil of microorganisms. According to Hakim et al (1986) in areas with high rainfall nitrate loss is greater. Availability of nitrate in the soil is one of the factors that determine the rate of denitrification.  $NO_3$  very unstable on waterlogged soil conditions, which in a few days after flooding nitrate will be lost as N2O and N<sub>2</sub> through denitrification.

Observations on the  $40^{\text{th}}$  days showed nitrate levels in all treatment has increased, especially in urea and control with BNT value = 2.83. This is due to the availability of ammonium as the initial substrate to initiate the nitrification is the process in which ammonium (NH<sub>4</sub><sup>+</sup>) is converted to nitrite (NO<sub>2</sub>) and then nitrate (NO<sub>3</sub>). This process occurs naturally in the environment and is done by a group of bacteria Nitrosomonas and Nitrobacteria. While nitrate levels in the treatment of urea coated nitrification inhibitors are not as high as with urea and control. Nitrification inhibitors that have been used in the field can efficiently use fertilizer and can reduce N leaching and denitrification by providing N in the soil in the form of ammonium (NH<sub>4</sub><sup>+</sup>). Dicyandiamide (DCD) acts as an inhibitor of nitrification by inhibiting the process or the first stage of nitrification, oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) into nitrite (NO<sub>2</sub>) and inactivate the enzyme produced by nitrification bacteria that AMO (ammonia monooxygenase) (Jumadi et al, 2008),

Observations on 55th days showed high nitrate levels in all treatments decreased except for urea with BNT value = 4.09. This happens because two days before sampling is done only on the plot urea fertilization, so ammonium in the soil which is the primary substrate for the continuity of nitrification that will produce fairly high nitrate content in the soil. High nitrate levels in the treatment of urea. It is also due to the absence of additional nitrification inhibitors that neem seed extract and dicyandiamide (DCD), so that the rate of nitrification running fast. Besides urea rapidly hydrolyzed in moist soil by the enzyme urease into ammonium within 2 to 3 days to make the availability of ammonium  $(NH_4^+)$  high, in the presence of ammonium then initial substrate for the ongoing nitrification. Where ammonium  $(NH_4^+)$  will be converted into nitrite  $(NO_2)$ by bacteria Nitrosomonas hereinafter nitrite (NO<sub>2</sub>) is converted into nitrate (NO<sub>3</sub>) by Nitrobacter bacteria. The amount of nitrate in the control treatment is also likely to be high. This is because the microorganisms that decompose organic matter so that the availability of N-ammonium quite as substrate nitrification and there are many populations of nitrifying bacteria that quickly changed N-ammonium into nitrate (NO<sub>3</sub><sup>-</sup>) because the clay has good soil moisture, have humidity and pH 6.2 is suitable for bacterial nitrification activity. Based on the results of soil analysis at BPTP Maros, clayey loam soil pH ranged from 5.07 to 6.20. According to Purwanto (2009), the highest population of nitrification bacteria found in neutral to alkaline pH (6-8), below pH 5 nitrification decreased.

#### CONCLUSION

1. The average levels after applied of urea ammonium coated material nitrification inhibitors in the soil is highest in the treatment of urea coated neem 5% compared with the treatment of urea coated neem 2.5% and urea coated DCD 5%.

2. The average nitrate levels after applied of urea coated material nitrification inhibitor in soil is lowest in the treatment of membranous DCD 5% urea and urea treatment of coated neem 5% compared to coated neem 2.5%.

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