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The Effect of Urease Inhibitors Coated Urea on the Growth, Physiological Activities and Yield of Maize (*Zea mays* L.) in Inceptisol Jogonalan, Klaten

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ABSTRACT
An approach to minimize the loss of nitrogen (N) evaporating from urea is by inhibiting urease activity through urea coating with N- (n-butyl) thiophosphoric triamide (NBPT) and N- (n-propyl) thiophosphoric triamide (NPPT). The effect of urease inhibitor to minimize N loss has not been done in inceptisol soil. The purpose of this research was to study the effect of urease inhibitor coated urea on growth, physiological activity and yield of maize in Inceptisol Jogonalan, Klaten, Indonesia. The treatment was laid out in a Randomized Complete Block Design with 4 replications, which were N0 (without fertilizer), N1 (urea + NBPT 348 kg ha^{-1}) and N2 (urea + NBPT 278 kg ha^{-1}). All treated plants was fertilized once in one growing season 3 weeks after planting (WAP). The results showed that fertilization affected all observed variables. The effect of urea coated with inhibitor urease (NBPT+NPPT) compared with N0 (control) showed very optimum result, especially in N2 treatment. The urea coated with NBPT + NPPT (urease inhibitors) was more effective at lower dose of 278 kg ha^{-1} and tended to provide better results indicated by the growth and yield of maize in Inceptisol, Jogonalan, Klaten. The better growth and the higher yield of maize that was indicated by seed weight of 11.45 ton ha^{-1} and 100-seed weight of 37.75 g were obtained from N2 treatment as compared to other treatments. The hybrid maize (P35) had an optimum yield potential of 12.1 ton of dry pipes per hectare.

Keywords: Urease inhibitor, NBPT, NPPT, urea, corn, inceptisol.

INTRODUCTION

Nitrogen (N) is a major nutrient of plants that is mostly absorbed in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Although N is very abundant in nature, N content in soil solution is not more than 2% which is available for plants. Therefore, the additional input of N fertilizer, especially urea, is highly needed to improve the plant productivity. High N content of urea fertilizer (46%), affordable price and easy management are the good reasons to choose (Artola *et al*., 2011). Nitrogen is absorbed by maize during the growth period until seed maturation, so this plant requires the continuous availability of N on all stadia of growth until seed formation. Mulyani *et al.* (2001) stated that nitrogen fertilizer is very unstable, easily got lost by run-off, volatilization and leaching. This research examined N loss by volatilization, with highly mobile in the soil and there were urease enzymes which could increase N loss by volatilization. A high N loss rate affected low absorption efficiency. The efficiency of nitrogen uptake ranged from ± 20–40% (Kumar *et al*., 2000; Bouman *et al*., 2002). The condition results in a dose of urea on the cultivation of maize increased from time to time to get high maize productivity.

Increased N fertilization level is not able to overcome the problem of N absorption efficiency because the main problem in N fertilization is characteristic of N which is easily lost. This condition often occurs in soil types with good aeration potentials such as Inceptisol (Muyasir, 2012). An approach to maximize the absorption of nitrogen from urea is to reduce the rate of nitrogen loss through inhibition of urease activity. The inhibitory process of urease activity may be carried out by coating urea using N- (n-butyl) thiophosphoric triamide (NBPT) and N-Propyl-Thiophosphoric-Triamide (NPPT). According to

Medina & Radel (1988), NBPT and NPPT have the molecular formula of $C_4H_{14}N_3PS$ that is a structural analogue urea. This compound serves as a urease inhibitor which is known to reduce the nitrogen loss evaporated from urea in the form of ammonia (NH_3).

Field experiments were conducted on Inceptisols as a dryland with low fertility and low organic matter content (Suriadikarta *et al*., 2002). This posed a challenge for maize cultivation at Inceptisol because it will require more N fertilizers than that in other soil types. Nursyamsi and Suprihati (2005) in their research results stated that the demand for N fertilizer in Inceptisol is higher than that in Oksisol and Andisol. At present, the dose of urea fertilization in maize reached ± 350 to 450 kg per season (Akil, 2009; Suratmini, 2009; Soares *et al*., 2012; Nurmegawati, 2015). Therefore, the objectives of this research were (1) to determine the effect of urease inhibitor coated urea in in Inceptisol (2) to know the effect of urease inhibitor coated urea on growth, physiological activity and crop yield.

MATERIALS AND METHODS

The research was carried out in Tangkisanpos village, Jogonalan Sub-district, Klaten Regency, from May to September 2016. The primary materials used were maize hybrid varieties of pionner (P35) and urease inhibitor coated urea. Historically, this land is often used for cultivating corn and tobacco. The result of soil analysis showed that N nutrient status was very low (0.08%) compared to P (4.6 mg kg^{-1}) and K (11.16 cmol kg^{-1}). The microclimate environment on which the research and observation took place showed a quite high of rainfall ranging from 432 mm $month^{-1}$, air humidity ranging from 68–72%, and temperature ranging from 26°C–31°C. Overall environmental conditions of research site was very supportive and optimal enough for the cultivation of maize crops.

A field experiment were designed using Randomized Complete Block Design (RCBD), single factor, with four blocks as replications. The single factor was the fertilization dose of urea coated with NBPT+NPPT, treatment without fertilizer, dose of 348 kg ha^{-1} and dose of 278 kg ha^{-1}. Twelve plots used in this present experiment had each plot with an area of 12 m^2.

Observations were done on several variables: soil physicochemical characteristics, plant physiological activity, and growth and yield of maize. The chlorophyll content was quantified based on Hendry and Grime method (1993) *cit*. (Lestari *et al*., 2008). Observation on nitrate reductase activity was based on Hartiko method (1991). Observation on transpiration rate and photosynthetic rate applied Portable Photo-synthesis System Li-6400. The data obtained were subjected to analysis of variance (ANOVA) followed by least significant difference (LSD) test to compare among means. All analyses were performed by the General Linear Model Procedure (PROC GLM) (SAS Institute, 1990).

RESULTS AND DISCUSSION

Physicochemical Characteristics of Inceptisol

Physicochemical characteristics test was performed by sampling in a 0–20 cm deep soil layer compositely at the experiment site with the result shown in Table 1. The analyis results provided information that Inceptisols in Jogonalan Klaten had a sandy soil texture with neutral pH and low N content. Thus, the application of urease inhibitor coated urea was expected to increase the maize productivity in the area.

The lowest value of soil total N content after treatment was shown by N0 but it was not significantly different compared to others (Table 2). The decrease

Table 1. Physicochemical characteristics of Inceptisol in Jogonalan, Klaten before treatment

	Inceptisol	Method
Soil texture		
Sand (%)	38	Hydrometer
Silt (%)	45	Hydrometer
Clay (%)	17	Hydrometer
C-Organic (%)	0.56	Walkly & black
Soil pH	6.66	pH meter 1 : 5
Total N (%)	0.07	Kjeldahl
CEC (cmol (+) kg^{-1})	11.68	Distillation

Sources: soil analysis by BPTP Yogyakarta.

Table 2. Soil total N content (%) after treatment

Treatment	Total N (%)
Without Fertilizer	0.05 a
Dose of 348 kg ha^{-1}	0.07 a
Dose of 278 kg ha^{-1}	0.07 a

Remark: Means in column followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). Without fertilizer (N0), dose of 348 kg ha-1 (N1) and dose of 278 kg ha-1 (N2)

Table 3. Shoot and root fresh weight (FW) and dry weight (DW) (g) and root volume (mL) of maize as affected by treatment

Treatment	Root		Shoot		Root volume
	FW	DW	FW	DW	
Without Fertilizer	65 b	12.00 b	118.75 b	41.09 b	17.50 b
Dose of 348 kg ha^{-1}	135 ab	21.85 a	147.00 a	47.14 a	35.00 a
Dose of 278 kg ha^{-1}	166 a	24.06 a	149.50 a	48.48 a	38.75 a

Remark: Means in each column followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). Without fertilizer (N0), dose of 348 kg ha^{-1} (N1) and dose of 278 kg ha^{-1} (N2)

Table 4. Chlorophyll content ($mg.g^{-1}$) and Nitrate Reductase Activity (μmol $NO_2^-.g^{-1}.hour^{-1}$) of maize as affected by treatment

Treatment	Chlorophyll Content	Nitrate Reductase Activity
Without Fertilizer	0.94 b	1.69 a
Dose of 348 kg ha^{-1}	1.06 a	2.53 a
Dose of 278 kg ha^{-1}	0.98 a	2.20 a

Remark: Means in column followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). Without fertilizer (N0), dose of 348 kg ha^{-1} (N1) and dose of 278 kg ha^{-1} (N2)

in soil N content indicated that untreated maize crop used existing N residuals in the soil to meet their nutrient needs. At the same time, the soil total N content as affected by N1 and N2 showed the same value 0.07%. These same values suggested that the addition of urease inhibitor coated urea was well utilized so that the residual N was not much in the soil. However, N2 had a more positive tendency since the lower dose was able to produce the same value compared to N1.

The response from the maize growth related to fresh and dry weight of shoot and root showed that the urease inhibitor coated urea had more significant effect compared to untreated plant. As presented in Table 3, N2, a lower dose in urease inhibitor coated urea treatment gave better result than N1 which was indicated by root fresh weight and dry weight that was in line with the highest value in root volume.

The fresh weight of shoot and root was observed to determine the status of water in the plant body. The water status of a tissue or whole plant body might change as the plant age increased and inconstant environment occurred (Goldsworthy & Fisher, 1992). Meanwhile, dry weight was also important to observe because it was able to describe all events in plant's life cycle as indicated by the accumulation of assimilates (Sitompul & Guritno, 1995). Dry matter is the fixed weight of the heated sample at 105 ºC in the oven until all water evaporates (AOAC, 1970).

The results of urea application coated with NBPT+NPPT showed much better than control (N0) on plant physiological activity (Table 4 and 5). Comparison between N1 and N2 statistically showed similar results but better value trend was found in N1. In contrast to previous data at shoot and root, N2 was much better. It was thought that higher N greatly affected chlorophyll content and NRA, which were correlated to the photosynthetic and transpiration rate.

Sutedjo and Kartasapoetra (1990) described about the availability of soil N elements that can produce more protein. The higher the nitrogen, the faster the synthesis of carbohydrates would be to be converted into protein. Moreover, the increased protoplasm of protein in the plant body will increase N levels in plant tissues. According to Latifa (2009), assimilation of N into organic molecules depends on the reduction of NO_3^- by the enzyme nitrate reductase in the plant

tissue. Nitrate reduction that must occur before amino acid production requires electrons. The main donor of these electrons is Nicotinamide Adenine Dinucleotide (NADH), which is the result of photosynthesis so that the value of ANR is also affected by the photosynthetic rate. The experimental results indicated statistically similar results betwen N1 and N2 but better NRA tendency in N1 treatment (Table 4) which was similar to the photosynthetic rate (Table 5). The better leaf chlorophyll content tendency found in N1 treatment was also aligned with the NRA value and the photosynthetic rate. Furthermore, the transpiration rate supported the entry of CO_2 which was the main ingredient of the plant to undergo photosynthesis. The better values tendency of transpiration rate was also obtained from N1 treatment.

Generally, the statistical results of plant growth showed that there was an insignificant difference between NBPT+NPPT coated urea treatment affecting the plant growth (Figure 1). However, both treatments (N1 and N2) provided better results compared to control (N0).

Based on the result of ANOVA, total N content on roots, shoots, and leaves was aligned with the leaf chlorophyll content in which N1 showed higher N content than N2. Although, total N content in root, shoot, and leaf were lower in N2 than that in N1, N content in seed of N2 had the same value as the higher NBPT + NPPT-coated urea dose (N1). Cancellier *et al*., (2016) explained that N-losses suppressed by NBPT + NPPT caused N content to be accumulated in stumps and grains, and yields of stumps and grains.

The hybrid maize (P35) had an optimum yield potential of 12.1 tons of dry pipes per hectare. The effect of inhibitor urease coated urea (NBPT+NPPT)

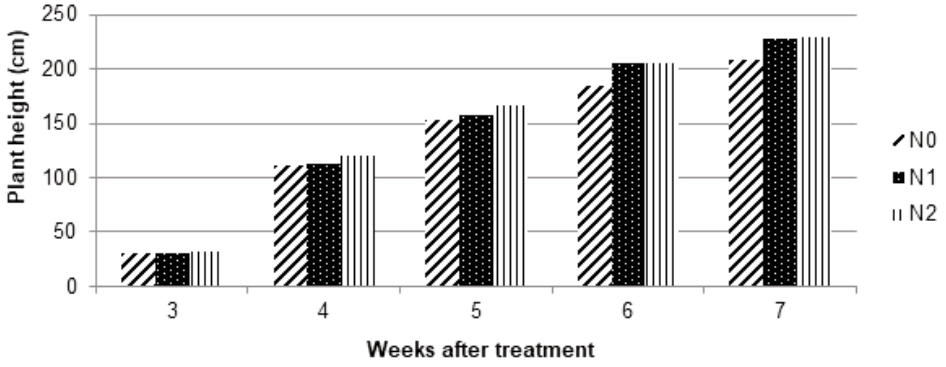

Figure 1. Plant height of maize (Zea mays) grown in Inceptisol at Jogonalan, Klaten as affected by treatment

Table 5. Transpiration rate ($mg.g^{-1}$) and photosynthetic rate ($\mu mol\ NO_2^-.g^{-1}.hour^{-1}$) of maize as affected by treatment

Treatment	Transpiration rate	Photosynthetic rate
Without Fertilizer	158.00 b	0.93 b
Dose of 348 kg ha^{-1}	168.00 a	2.80 a
Dose of 278 kg ha^{-1}	165.50 a	2.64 a

Remark: Means in column followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). Without fertilizer (N0), dose of 348 kg ha^{-1} (N1) and dose of 278 kg ha^{-1} (N2)

Table 6. Total N content of root, shoot, leaf, and seed of maize (%) as affected by treatment

Treatment	Total N content			
	Root	Shoot	Leaf	Seed
Without Fertilizer	0.39 b	0.38 b	1.69 b	0.56 b
Dose of 348 kg ha^{-1}	0.63 a	0.52 a	2.13 a	0.79 a
Dose of 278 kg ha^{-1}	0.52 ab	0.51 a	1.97 ab	0.79 a

Remark: Means in each column followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). Without fertilizer (N0), dose of 348 kg ha^{-1} (N1) and dose of 278 kg ha^{-1} (N2)

Table 7. Seed weight ha^{-1} (ton), 100-seed weight (g), and N absorption efficiency (kg kg^{-1})

Treatment	Seed weight per ha	100-seed weight	N Absorption Efficiency
Without Fertilizer	5.23 b	21.50 b	2.98 b
Dose of 348 kg ha^{-1}	10.28 ab	34.00 ab	3.57 a
Dose of 278 kg ha^{-1}	11.45 a	37.75 a	4.15 a

Remark: Means in column followed by the same letter are not significantly different according to the LSD test (P < 0.05). Without fertilizer (N0), dose of 348 kg ha-1 (N1) and dose of 278 kg ha-1 (N2)

compared with control showed very optimum result, especially in N2 treatment (Table 7). The highest yield of maize for seed weight per ha^{-1} was 11.45 tons ha^{-1} and 100-seeds weight was 37.75 g as affected by N2. The highest yield of maize was also affected by the N absorption efficiency (kg kg^{-1}) that the highest value was also found in N2. The same research results showed by Akil (2013), that in dose of 225 until 350 kg ha^{-1} yield of maize for seed weight per ha^{-1} was 11.51 ton ha^{-1} which was also in inceptisol soil.

Overall, the effect of urease inhibitor coated urea (NBPT + NPPT) was efficacious compared to control. However, NBPT + NPPT in high and low doses also showed different results. The effect of urea coated with NBPT + NPPT was more effective at lower dose. It was in line with the statement of Antasari *et al*., (1996) and Rawluk *et al*., (2001) that the use of urease inhibitor coated urea was more efficient at low concentration. Urease inhibitor coated urea made urea more stable and have slow release. Urea inhibitors were able to inhibit urea loss up to two days and reduce the accumulation of evaporation to 18% when compared to control. Cancellier *et al*., (2016) also suggested that the appropriate dose was the combination of urea with NBPT as much as 200 kg ha^{-1} that was capable to yield 16% bigger and greater N accumulation compared to control. This was in accordance with the results shown through this present study. The application of urea coated with NBPT at a dose of 278 kg ha^{-1} showed better results than control and the higher dose of urea coated with NBPT + NPPT (348 kg ha^{-1}).

CONCLUSIONS

The best treatment on this present study was NPPT + NPPT-coated urea with a lower dose of 278 kg ha^{-1} (N2). The better growth as well as the higher yield of maize were indicated by seed weight of 11.45 ton ha^{-1} and 100-seed weight of 37.75 g which were obtained from N2 treatment as compared to other treatments.

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