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EFFECTS OF NITRIFICATION INHIBITOR WITH ORGANIC MANURE AND UREA ON PROTEIN AND MINERAL CONTENTS IN GRAIN OF *ORYZA SATIVA* CV. MR219 CULTIVATED IN ACID SULPHATE SOIL

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Key words: Nitrification inhibitor, Dicyandiamide, Organic manure, MR219 rice, Acid sulphate soil

Abstract

Several approaches to improve grain nutritive values involve in increasing seed accumulation of protein and micronutrients in rice. Therefore, a study was conducted to select a suitable combination of dicyandiamide (DCD) with organic manure (OM) and urea to improve protein and mineral content in rice grain of MR219. The protein (9.07-12.50%) and Ca, Mg, Zn, Cu, Fe and Mn concentrations increased from 1.92 to 21.05, 3.56 to 18.25, 2.25 to 20.22, 9.14 to 25.66, 3.34 to 27.20 and 5.17 to 23.86%, respectively due to the application of DCD with urea and OM. Moreover, the highest content of protein in grain was obtained for the application of DCD with urea and oil palm compost (OPC). Iron and Mn contents were also highest for DCD with urea and OPC, but Ca, Mg, Zn and Cu contents were highest for DCD with urea and poultry dung (PD) and kept similarity with DCD with urea and OPC.

Introduction

Populations residing both in developed and developing countries consume cereals as primary food component. Cereals are inherently low in minerals and protein contents. Poor grain nutritive value of cereals is an important reason of widespread protein and micronutrients malnutrition among populations eating rice as staple food. Rice provides 50 to 85% of daily energy source and is consumed in large amounts. Therefore, a little increase in grain nutritive value of rice will produce a cumulative effect on total nutrient intake. Deposition of protein, iron (Fe) and zinc (Zn) in rice grains depends on the interrelated metabolic pathways involved in uptake of N, Fe and Zn from soil, their transport to source tissues, such as culms and leaves and mobilization and/or remobilization to developing grains (Mahmoud *et al.* 2008). External fertilization for N in form of *Azolla*, rice straw or urea, has been reported to increase biomass of rice plants and also to affect grain quality traits such as total grain protein content (Perez *et al.* 1990). Therefore, the possible approaches to improve grain nutritive values involve with increasing seed accumulation of protein and micronutrients. Application of chemical fertilizer combined with OM is favourable to the nutrient accumulation by rice during the middle and late growing period (Ming-Gang *et al.* 2008). Nitrogen fertilizer promoted the accumulation of protein, decreased the accumulation of amylose in grain, and enhanced gel consistency of brown rice. The appropriate N fertilizer management could increase micronutrient contents in grain and improve nutrition quality of rice (Hu-Lin *et al.* 2007). Although N fertilization with organic and inorganic has been known to increase protein and

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biomass production in rice, yet we have little information regarding the effect of nitrification inhibitor with OM and urea on protein and mineral deposition in the grains of MR219 rice produced in acid sulphate soil. Hence, the objective of this study was to select a suitable combination of DCD with OM and urea to improve protein and mineral content in rice grain of MR219.

Material and Methods

This experiment was conducted at experimental farm (Ladang 10) of Universiti Putra Malaysia, Serdang, Selangor, Malaysia. It is geographically situated at 3.30^0N latitude and 101.50^0E longitudes of Peninsular Malaysia. Rice *(Oryza sativa* L.*)* cultivar MR 219 was used. The experiment was organized in a randomized complete block design with three replicates. Treatments comprised four N source: 100% N (urea) of recommended dose from urea, 75% N (urea) + 25% N (RS), 75% N (urea) + 25% N (PD) and 75% N (urea) + 25% N (OPC), and two levels of nitrification inhibitor: without inhibitor (NoNI) and with inhibitor (NI). A 20 litre plastic bucket was filled with 14 kg air-dry soil. Urea, triple super phosphate (TSP) and muriate of potash (MoP) were used as N, P and K fertilizers, respectively. Each treatment supplied with P (30 kg/ha) and K (60 kg/ha) before rice transplanting. Organic manure: i.e. RS, PD and OPC were applied as per treatments one week before the final land preparation. Phosphorous from TSP and K from MoP were applied before one day of transplanting. Nitrogen from urea was used as top dressing in three equal splits at the time of TSP and MoP application, maximum tillering stage and at booting stage of crop growth. Dicyandiamide was applied at the rate of 15% of N with urea. The 15 days old seedlings were transplanted into bucket with two hills in each bucket and three seedlings per hill. The bucket was flooded at two days before rice transplanting, and rice management was similar as that in the paddy field. Different intercultural operations and plant protection measures were conducted following standard practices (MARDI 2002).

At maturity, both hills from each pot were used for collecting grain sample. All the sample panicles were hand-threshed and filled grains were separated from unfilled grains. After sun-dried at 14% moisture content sample grain was de-hulled and kept at ambient condition. Grain samples were dried in an oven at 65^0C for 48 hrs and then ground by a grinding machine to pass through a 20 mesh sieve and stored in small paper bags into a desiccator. Nutrient content in each sample was determined by H_2O_2-H_2SO_4 digestion (Ohyama *et al.* 1991) using a Kel Plus auto N analyzer for N and spectrophotometer for Ca and Mg. Iron Mn, Cu and Zn concentrations were measured with an ICP-MS (Agilent 7500a). The protein content in grain was determined by the micro-Kjeldahl method (Fei *et al.* 2008). Protein content = (6.25 × nitrogen content/dry matter weight) × 100%.

Data were subjected to a two-way analysis of variance (ANOVA) (source and nitrification inhibitor) using the PROC GLM function of the SAS statistical programme (SAS Institute 1996). When there was a significant treatment effect, means were compared using DMRT. Treatment comparisons were deemed significant at $p < 0.05$.

Results and Discussion

The extent of protein content in grain ranged from 7.62 to 8.29% with DCD and 7.49 to 8.00% with N sources (Fig. 1). Dicyandiamide gave higher protein content compared to that without DCD with the N source. The highest protein content was recorded in urea + PD with DCD (8.40%), which were statistically similar to that of DCD with urea + OPC (8.29%) and the lowest value 7.29% was in urea alone. The findings of this study was supported by other workers who showed that the application of chemical fertilizer combined with OM increased N uptake and increased the N use efficiency of rice (Ming-Gang *et al.* 2008), as a result promoted the accumulation of protein in grain. It was shown that the protein concentrations in polished rice increased with the increase in N rates (Li *et al.* 2007, Yuan *et al.* 2014).

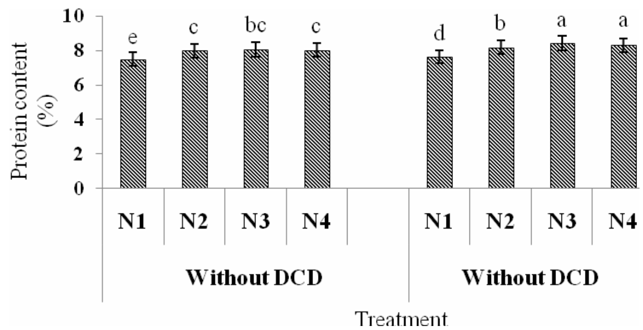

Fig. 1. Effects of DCD with organic manure and urea on protein content of rice cultivar MR219. Vertical bars represent ± standard error of mean. N_1 = 100% N (urea), N_2 = 75% N (urea) + 25% N (RS), N_3 = 75% N (urea) + 25% N (OPC) and N_4 = 75% N (urea) + 25% N (PD); DCD = Dicyandiamide, RS = Rice straw, OPC = Oil palm compost and PD = Poultry dung.

There was a significant interaction effect of DCD and N source on the Ca and Mg content in grain of MR219 rice (Table 1). Across the N source, higher value of these elements was recorded in the presence of DCD than the absence of DCD. As a result, the content of Ca (0.138 mg/g) and Mg (0.149 mg/g) in grain were the highest due to the application of DCD with urea + PD (0.138 mg/g) followed by DCD with urea + OPC (0.131 and 0.144 mg/g, respectively) and the lowest content of these elements were recorded for urea alone (0.114 and 0.126 mg/g, respectively).

Table 1. Effects of DCD with organic manure and urea on protein content in grain of MR219 rice.

N source	Ca		Mg	
	Without DCD	With DCD	Without DCD	With DCD
	mg/g			
N_1	1.14 ± 0.003e	1.16 ± 0.003de	1.26 ± 0.003e	1.30 ± 0.004de
N_2	1.22 ± 0.003cd	1.25 ± 0.004bc	1.34 ± 0.004cd	1.41 ± 0.005bc
N_3	1.27 ± 0.004bc	1.38 ± 0.005a	1.38 ± 0.005bc	1.49 ± 0.004a
N_4	1.26 ± 0.005bc	1.31 ± 0.002b	1.37 ± 0.005bc	1.44 ± 0.004ab
CV (%)	2.59		2.98	

Means followed by the same letter within the same column are not significantly different ($p > 0.05$) using DMRT.

The extent of Zn and Cu content ranged from 40.36 to 47.56 and 16.40 to 19.10 mg/kg over DCD and 39.56 to 42.20 and 15.20 to 18.00 mg/kg over N source (Table 2). The content of these elements significantly varied with and without DCD showing that DCD had higher content compared to no DCD. The interaction of DCD with urea + PD showed the maximum Zn (47.56 mg/kg) and Cu (19.10 mg/kg) keeping similarity with urea + PD (46.43 mg/kg) for Zn and DCD with urea + OPC (18.40 mg/kg) for Cu (Table 2).

Dicyandiamide with urea + OPC showed highest Fe (50.40 mg/ka) and Cu (43.60 mg/ka) contents which were statistically similar to DCD with urea + PD (49.20 mg/ka) for Fe and with

DCD with urea + RS (42.00 mg/ka) and urea + RS (41.60 mg/ka) for Mn. The lowest contents (39.60 and 35.20 mg/ka) of Fe and Mn in grain were estimated for urea alone (Table 3). These results are supported by the work of Zhang *et al*. (2001) who found that soil organic matter exert a significant and direct impact on the availability of Zn, Fe and Mn but has little influence on the availability of soil Cu. In addition, the interaction of other soil macro- and micro-nutrients also affected micro-nutrients uptake by crops (Aulakh and Malhi 2005). Similarly, soil organic matter content increases the availability of Fe and Zn in rhizosphere, which in turn enhances the uptake, translocation and redistribution of Fe/Zn into rice grains (Chandel *et al.* 2010). It has been observed that combined application of organic and inorganic fertilizers promoted the transfer of nutrients to the grains (Yang *et al.* 2004).

Table 2. Effects of DCD with organic manure and urea on Zn and Cu contents in grain of MR219 rice

N source	Zn		Cu	
	Without DCD	With DCD	Without DCD	With DCD
	mg/kg			
N_1	39.56 ± 1.07e	40.36 ± 1.25de	15.20 ± 0.40e	16.40 ± 0.45d
N_2	43.53 ± 1.30c	44.36 ± 0.74bc	17.20 ± 0.42cd	17.60 ± 0.40bc
N_3	46.43 ± 1.29ab	47.56 ± 1.50a	18.00 ± 0.41bc	19.10 ± 0.79a
N_4	42.20 ± 1.20cd	44.06 ± 1.92bc	17.60 ±0.44bc	18.40 ± 0.77ab
CV (%)	3.13		3.19	

Means followed by the same letter within the same column are not significantly different (p > 0.05) using DMRT.

Table 3. Effects of DCD with organic manure and urea on Fe and Mn contents in grain of MR219 rice.

N source	Fe		Mn	
	Without DCD	With DCD	Without DCD	With DCD
N_1	39.60 ± 1.30f	40.80 ± 0.80ef	35.20 ± 1.20e	36.80 ± 1.21de
N_2	42.40 ± 1.40de	43.60 ± 1.35d	41.60 ± 1.26ab	42.00 ± 1.60ab
N_3	46.40 ± 1.12c	49.20 ± 1.10ab	38.80 ± 1.21cd	40.00 ± 1.12bc
N_4	47.20 ± 1.60bc	50.40 ± 1.44a	42.40 ± 1.61a	43.60 ± 1.15a
CV (%)	2.87		2.94	

Means followed by the same letter within the same column are not significantly different (p > 0.05) using DMRT.

Combined application of DCD along with OM and urea improve protein and mineral content in grain over the application of urea alone. Among the OM, OPC along with urea and DCD is most effective for higher protein and mineral content in grain. These results suggest that the combination of DCD with OPC and urea may be the most potential combination to improve the protein and mineral contents in rice grain.

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