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Residual Effect of Nitrogen Fertilization on Nitrous Oxide Flux and Yield of Three Cowpea Varieties (*Vigna unguiculata* L.) in Rainfed Rice Fields

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ABSTRACT

Nitrogen fertilizer use in rainfed rice fields is generally less efficient, only part of N is taken up by plants for their gowth and other N is lost and fixed by soil particles. Nitrogen loss in the form of nitrous oxide can reduce N fertilizer use efficiency and contribute to the increase of atmospheric greenhouse gases emission. The field experiment was conducted to determine the residual effect of N fertilizer on nitrous oxide (N_2O) flux and yield of some cowpea varieties (*Vigna unguiculata*) in rainfed rice fields. The experiment was arranged in a factorial randomized block design with three replicates. The first factor was three cowpea varieties (KT 9, KT 6, KT 3), while the second factor was four levels of residual inorganic N fertilizer (0, 90, 135, 180 kg N ha^{-1}). The variables measured were N_2O fluxes, grain yield, biomass weight, total N content in soil before planting cowpea, available N in soil after harvesting cowpea. Residual N fertilizer increased significantly N_2O emission from cowpea cropping. Nitrous oxide emission from plots grown with cowpea variety of KT 9, KT 6, and KT 3 ranged 0.42-0.69, 0.30-2.64, and 0.09-2.19 kg N_2O ha^{-1}, respectively. N losses from soil grown with KT 9 was lower than those in plots grown with other varieties. Residual effect of N fertilizer increased available N in soil as much as 11.6-82.3% (KT 9), 7.6-30.6 % (KT 6), and 9.6-67.9% (KT 3), recpectively. Residual effect of N fertilizer increased significantly grain yield of KT 9, KT 6, and KT 3 varieties as much as 45.7-111.8%, 79.8-89.3%, and 6.9-25.4%, respectively.

Keywords: Cowpea, nitrous oxide flux, rainfed lowland areas, residue of N fertilizer

ABSTRAK

Penggunaan pupuk nitrogen pada lahan tadah hujan secara umum kurang efisien, sebagian N diserap untuk pertumbuhan tanaman dan sebagian yang lain hilang dan terjerap pada partikel koloid tanah. Nitrous oksida merupakan salah satu bentuk kehilangan N yang dapat menurunkan efisiensi pemupukan N and meningkatkan emisi gas rumah kaca ke atmosfer. Percobaan lapangan dilakukan untuk mempelajari efek residu pemupukan N terhadap nitrous oksida dan hasil panen dari beberapa varietas kacang tunggak (*Vigna unguiculata*) pada lahan tadah hujan. Percobaan disusun dalam rancangan acak kelompok faktorial dengan tiga ulangan. Faktor pertama adalah tiga varietas kacang tunggak (KT 9, KT 6, KT 3) dan faktor kedua adalah empat level residu pupuk anorganik N (0, 90, 135, 180 kg N ha^{-1}). Variabel yang diamati adalah fluks N_2O, hasil biji dan biomassa tanaman kacang tunggak, kandungan total N di dalam tanah sebelum tanam, dan kandungan N-tersedia di dalam tanah setelah panen. Residu pupuk N secara signifikan meningkatkan emisi N_2O pada lahan yang ditanami kacang tunggak. Emisi N_2O pada lahan yang ditanami varietas KT9, KT6 dan KT3 secara berturut-turut berkisar antara 0,42 – 0,69; 0,30 – 2,64; dan 0,09 – 2,19 kg N_2O ha^{-1}. Kehilangan N pada lahan yang ditanami KT 9 lebih rendah dibandingkan pada lahan yang ditanami varietas lain. Efek residu pemupukan N meningkatkan N-tersedia di dalam tanah secara berturut-turut sebesar 11,6 – 82,3% (pada KT 9); 7,6 – 30,6% (pada KT 6); dan 9,6 – 67,9% (pada KT 3). Efek residu pemupukan N secara signifikan meningkatkan hasil biji kacang tunggak varietas KT 9, KT 6, dan KT 3 secara bertutut-turut sebesar 45,7 – 111,8%; 79,8 – 89,3% dan 6,9 – 25,4%.

Kata kunci: Kacang tunggak, lahan tadah hujan, nitrous oksida, residu pupuk N

INTRODUCTION

Optimizing rainfed rice fields contributes significantly on stabilization of food security. There are several challenges in optimizing such lands, *i.e.* erratic water availability, low soil fertility, low plant productivity, and vulnerable to climate change impacts. The integrated efforts could optimize rainfed rice fields, *i.e.* selection of adaptive plant species, crop rotation, and proper application of cultivation technology.

Application of organic and inorganic fertilizers, including fertilizer containing nitrogen (N) is needed to support plant growth. The N fertilization plays an important role in improving soil fertility, enhancing crop yields (grains and biomass), and improving crop productivity (Ali *et al*. 2015). Inorganic N fertilizer is always applied every planting season either in wet season or dry season. Crops, especially rice generally only uses part of N from fertilizers, some of N then is lost and bound in the soil. The N loss from an inorganic N fertilizer can be in the form of nitrous oxide (Cassmann *et al.* 2002; Millar *et al.* 2018). Nitrous oxide (N_2O) is one of the greenhouse gases that contributes to global warming (Wang *et al*. 2012). Residual N fertilizer in soil can be utilized to grow the following crops in the next planting season, *e.g.* cowpea (*Vigna unguiculata* L.). On the other hand, the residue of N fertilizer can also be a source of N_2O emissions due to nitrification and denitrification processes in soil induced by microbial activity. Transformation of ammonia (NH_4^+) to nitrate (NO_3^-) via nitrification and denitrification processes produces secondary compounds such as N_2O (Chen *et al.* 2008; Signor and Cerri 2013).

The release of N_2O into the atmosphere is one of the N loss mechanisms from soil-plant system that leads to low fertilizer use efficiency in agricultural land. Nitrous oxide (N_2O) has higher global warming potential than methane (CH_4), namely 310 times CO_2 (IPCC 2014). Nitrous oxide emission generally increases with increasing N input, N fertilization, and decomposition of residual organic matter in soils (Jeuffroy *et al*. 2013; Wang *et al.* 2015). The N_2O production in the rhizosphere is translocated into plant tissues and is released through opened stomata to the atmosphere. The main factors affecting N_2O production in soil are plant biomass (Abalos *et al.* 2017), soil nitrate content, soil moisture content (Baruah *et al*. 2010), nitrogen fertilizer application (Millar *et al.* 2018), and plant type (Abalos *et al.* 2017). Application of N fertilizer with high rate tends to increase nitrous oxide flux (Millar *et al.* 2018). Plant species with deeper root penetration and larger biomass show large N uptake and N_2O flux (Abalos *et al.* 2017).

Cowpea (*Vigna unguiculata* L.) is a tolerant and adaptive legume crop against drought stress, moderately shade tolerant, resistant to pests, and adaptive to soils with low fertility (Oke and Eyitayo 2010; Rahmadani and Sunarlim 2013). Cowpea is multifunctional crop, among others as cover crop, weed controller, and erosion controller (Valenzuela and Smith 2002 *in* Rahmadani and Sunarlim 2013). Compared with soybean and mung bean, cowpea is relatively less intensively cultivated by farmers. Similar to other legumes, cowpea is a source of food with high protein content (Rahmadani and Sunarlim 2013) and important source of animal feed. At a demonstration plot scale, 100,000 hectares of land can produce 50,000 tons of cowpea seeds with productivity of 2 Mg ha^{-1} (Sumarno and Manwan 1990). As animal feed, cowpea contains 195 g kg^{-1} protein, 40.9 g kg^{-1} fat, 180 g kg^{-1} fiber, 448.3 g kg^{-1} free N (Lizhi 1994).

Cowpea is commonly cultivated in rice fields after planting season of rice. Besides utilizing N_2 from the atmosphere, cowpeas can utilize the residue of N fertilizer in soil for their growth. Cowpea may fix N from the atmosphere of more than 200 kg N ha^{-1} and accumulate N residue in soil of more than 92 kg N ha^{-1} (Rusinambodzi *et al. cit* Kyei-Boachen *et al*. 2017). Symbiosis between cowpea roots and *Rhizobium* could fix nitrogen from the atmosphere as much as 80-90% of plant requirement (Rahmadani and Sunarlim 2013). Therefore, the study was conducted to determine the residual effect of N fertilizer on nitrous oxide emission and yield of cowpea grown in rainfed lowland.

MATERIALS AND METHODS

A field experiment was conducted at Jakenan Experimetal Station of Indonesian Agricultural Environment Research Institute in Pati, Central Java during dry season of 2017. The experiment was located at 111°10' E and 6°45' S with the altitude of 12 m above sea level. The soil in the experimental site was generally characterized by deficiency of essential nutrients as presented in Table 1.

The experiment was arranged in a factorial randomized block design with three replicates. The first factor was cowpea variety that consisted of KT 9, KT 6, KT 3; while the second factor was residual N fertilizer that consisted of 0, 90, 135, 180 kg N ha^{-1}. Soil tillage was conducted in each plot with the size of 5 m x 6 m. Two seeds of cowpea per hole were planted with spacing of 30 cm x 20 cm in each plot. The fertilizers of P and K were

Table 1. Status of soil nutrients at experimental site.

Soil parameter	Method	Value	Criteria[1)]
pH-H_2O (1:2.5)	pH meter electrode	6.13	Slightly acid
pH-KCl (1:2.5)	pH meter electrode	3.23	
Electrical conductivity (dS m^{-1})	Conductometer	9.57	Very high
Total N (%)	Kjehdal	0.06	Very low
Organic C (%)	Walkley & Black	0.88	Very low
Available P (ppm P)	Olsen	22.56	Very high
Available K (ppm K_2O)	Morgan-Wolf	16.03	Very low
Cation exchange capacity (cmol/kg)	Extraction of NH_4OAc pH 7	11.55	Low
Exchangeable K (cmol/kg)	Extraction of NH_4OAc pH 7	0.05	Very low
Exchangeable Na (cmol/kg)	Extraction of NH_4OAc pH 7	1.26	Very high
Exchangeable Ca (cmol/kg)	Extraction of NH_4OAc pH 7	0.51	Very low
Exchangeable Mg (cmol/kg)	Extraction of NH_4OAc pH 7	0.64	Low

1) Source: Eviati and Sulaeman (2012)

applied at seven days after germination (DAG) with the dosage of 100 kg SP-36 ha^{-1} and 100 kg KCl ha^{-1}, respectively. Plant nurturing was done intensively. Rainfall distribution during cowpea growth is presented in Figure 1.

Some variables were measured, namely biomass weight, grain yield, nitrous oxide flux, total N in soil before planting, and available N in soil after harvesting cowpea. Available N in soil was determined by analyzing nitrate and ammonia using Morgan-Wolf method (Eviati and Sulaeman 2012). Nitrous oxide flux was measured at 5, 15, 30, 45, and 65 days after germination in all plots. Gas samples were taken using closed chambers of 40 cm x 20 cm x 40 cm that were laid between cowpea crops. Gas samples were taken using 10 mL syringe volume at 10, 20, 30 and 40 minutes after laying the chambers. Gas samples were injected into gas chromatography equipped with *electron capture detector (ECD).* After getting N_2O concentration, flux of N_2O was computed with a formula from IAEA (1993) *in* Setyanto *et al.* (2002) as follows:

$$E = \frac{Bm}{Vm} x \frac{\delta Csp}{\delta t} x \frac{V}{A} x \frac{273.2}{T + 273.2}$$

Note: ....(1)

E = flux of N_2O (μg m^{-2} day^{-1})
V = volume of chamber (m^3)
A = surface area of chamber (m^2)
T = air temperature inside chamber (°C)
dCsp/dt = rate of concentration change (ppb $minute^{-1}$)
Bm = molecule weight of N_2O
Vm = volume of gas in standard temperature and pressure (22.41 L)

Data was statistically analyzed using analysis of variance using SAS programme and continued with Duncan's Multiple Range Test (DMRT) at 5% significance level.

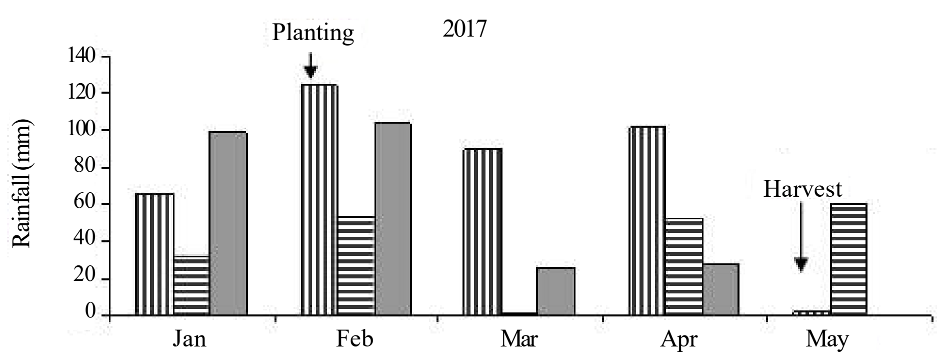

Figure 1. Rainfall distribution during the growth of cowpea at Jakenan Experimental Station, Central Java in 2017. ▥: first 10-days, ▤: second 10-days, ▩ : third 10-days.

RESULTS AND DISCUSSION

Residue of Total N in Soil

In general, N residues in topsoil with bulk density of 1.49 g cm^{-3} range from 2.4 to 5.3 Mg N ha^{-1} (Figure 2). Variation of N residue is influenced by indigenous N and N inputs from previous planting season. The total N residual content in soil varies significantly ($p < 0.0001$), which depends on N uptake by previous crops, organic matter in soil and its decomposition, N transformation in rhizosphere, and other factors that affect N availability in soil. The total N content in soil in plot without inorganic N fertilizer application generally was lower as much as 22-35.5% compared to that in soils applied with N fertilizer (Figure 2). The initial N content in soil in this study allows some to be mineralized and utilized by plants, and is lost in gaseous form such as N_2O. Nitrous oxide, which is one of greenhouse gases that can cause global warming and climate change, is an intermediate product of microbial processes of nitrification-denitrification.

Nitrous Oxide Flux

Figure 3 shows the flux of nitrous oxide (N_2O) from some cowpea varieties that utilize residue of N fertililization. In general, flux of N_2O was relatively high at 5 and 30 days after germination (DAG), and decreased at 65 DAG or at ripening phase. Flux of N_2O was relatively high at the beginning of cowpea growth phase, which is possible due to the high N release from rapid decomposition of organic matter in soil, whereas the plant N requirement is relatively low, which further causes high N lost and emission to the atmosphere (Singh *et al.,* 1995).

Flux of N_2O was relatively higher in plots grown with KT 6 and KT 3 varieties than in plots grown with KT 9 variety. Cowpea varieties have a genotype variation in releasing N_2O to the soil. Variety of KT 9 could release the lowest flux of N_2O to the atmosphere, indicating that this variety can utilize N from fertilizer efficiently. A mechanistic relationship between plant species and N_2O flux has not yet been established (Abalos *et al*. 2017). Improving efficiency of N fertilizer use can reduce N_2O emissions (Baruah *et al*. 2010). In plots grown with KT 6 variety, the residue of 90 kg N ha^{-1} resulted in higher N_2O flux than other residue rates. However in plots grown with KT 3 variety higher flux occurred on fertilizer residue of 135 kg N ha^{-1}. The production and release of N_2O from soil to the atmosphere is the result of interaction between roots of crop varieties and N availability in soil.

The positive interaction between cowpea varieties and N fertilizer residues influenced significantly N_2O emissions ($p < 0.0001$). Nitrous oxide emissions in plots grown with varieties KT 9, KT 6, and KT 3 ranged 0.42-0.69, 0.30-2.64, and 0.09-2.19 kg N_2O ha^{-1}, respectively (Table 1), which was equivalent to 0.26-0.44, 0.19-1.68, 0.05-1.39 kg N ha^{-1}. The rate of N fertilizer residue has increased significantly N_2O emissions from cowpea cropping. Application of N fertilizer activates nitrification and denitrification processes in soil that produce nitrous oxide (Legay *et al.* 2014). In plots without N application, the N_2O emission was lower

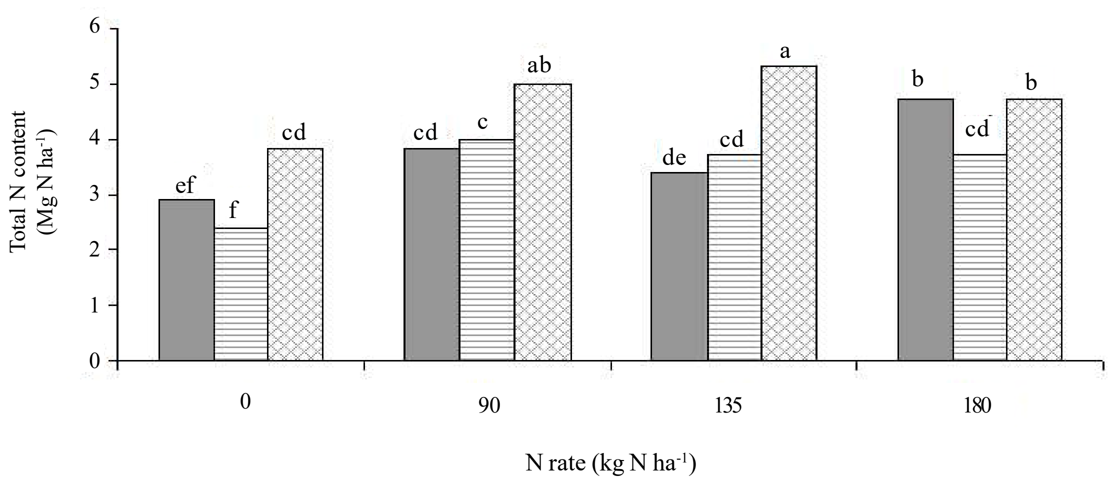

Figure 2. Total N content in soil after harvesting rice, before planting cowpea (Bars followed by the same letters are not significantly different according to DMRT at 5% level, coefficient of variance = 7.7625). ■ : KT 9, ▤ : KT 6, ▩ : KT 3

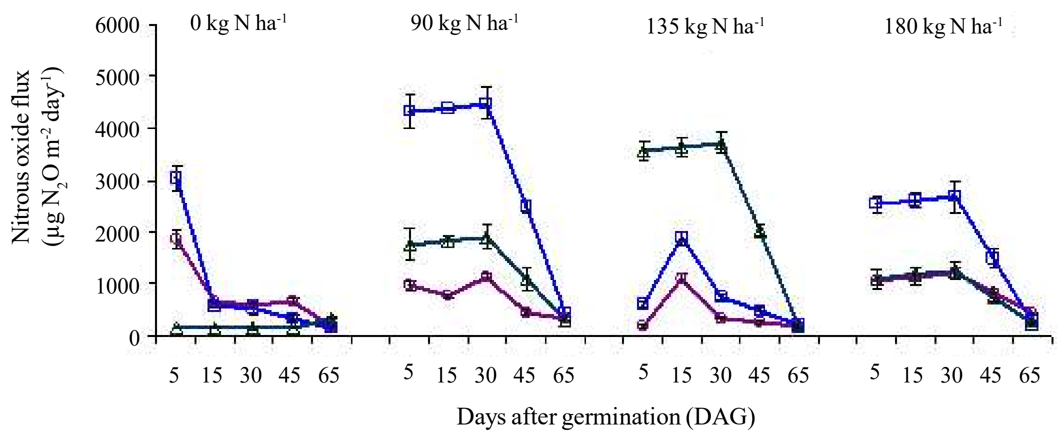

Figure 3. Nitrous oxide flux from three cowpea varieties with different amount of N fertilizer residue from previous planting season. —KT9, —KT6, —KT3

than in plots with N fertilizer residues. The N_2O released in no-N treatment is possible from mineralization of organic N in soil. According to Wang *et al.* (2012), accelerated mineralization of soil organic N and increased microbial activity can increase nitrate content and N_2O release into the atmosphere.

The highest N_2O emission was measured on the plots grown with cowpea variety of KT 6 treated with 90 kg N ha^{-1} (2.64 kg N_2O ha^{-1} season^{-1}), while the lowest emission of 0.09 kg N_2O ha^{-1} season^{-1} occured on the plots grown with KT 3 variety without residual N fertilizer (Table 2). A mechanistic relationship between plant species and N_2O flux has not yet been established (Abalos *et al*. 2017). In fact, legumes may produce their own N_2O through several pathways, *i.e.* (i) biological N_2 fixation, (ii) rhizodeposition of N inputs from plant roots into soil, and (iii) decomposition of crop residues and roots after harvesting plants and incorporating them into soil (Zhong *et al. cit* Jeuffroy *et al*. 2013).

The amount of available N in soil before harvest increased significantly (p <0.0004) with the increase of rates of N fertilizer residue (Table 2). During its growth, legumes absorb available N in the form of NO_3^- and NH_4^+. The highest amount of available N

Table 2. Available N and nitrous oxide emission from plots grown with different cowpea varieties and N fertilizer residues in rainfed rice field.

Cowpea variety	Residual effect of N fertilizer rate (kg N ha^{-1})	Available N in soil[1)] (kg N ha^{-1})	N_2O emission (kg N_2O ha^{-1})
KT 9	0	12.84 d	0.42 g
	90	14.33 cd	0.45 fg
	135	16.02 cd	0.61 ef
	180	23.41 a	0.69 e
KT 6	0	14.35 cd	0.30 g
	90	15.45 cd	2.64 a
	135	18.28 bc	1.06 d
	180	18.75 bc	1.57 c
KT3	0	12.95 d	0.09 h
	90	21.74 ab	1.10 d
	135	14.20 cd	2.19 b
	180	15.37 cd	0.71 e
Significance level		<0.0004	<0.0001
Coefficient of variance		15.45	10.06

The numbers followed by the same letters in the same column are not significantly different according to DMRT at 5% level. Soil available N was measured at maturity stage of cowpea crops.

in soil was measured on the plots grown with variety of KT 9 treated with 180 kg N ha^{-1}, while the lowest available N occured in the plots without N fertilizer residue. Residual N fertilizer increases available N in soil as much as 11.6-82.3% on the plots grown with KT 9 variety; 7.6-30.6% on the plots grown with KT 6; and 9.6-67.9% on the plots grown with KT 3, respectively. Cowpea variety has variance in N fixation from the atmosphere and N uptake from soil. According to Rahmadani and Sunarlim (2013), the number of root nodules of KT 6 variety is lower than other varieties such as KT 7 and KT 8, so that N fixation is also lower.

Cowpea Yield

Grain yield of cowpea from three varieties was affected significantly by residual N fertilizer in soil ($p < 0.05$). Residual N fertilizer in soil increased significantly grain yield of KT 9, KT 6, dan KT 3 varieties as much as 45.7-111.8%, 79.8-89.3%, and 6.9-25.4%, respectively. The amount of N residue of 90 kg N ha^{-1} was still able to increase significantly grain yield, however, the amount of N residue of more than 90 kg N ha^{-1} did not increase significantly grain yield of cowpea (Table 3). According to Ali *et al*. (2015), the increase of N fertilizer rate more than 120 kg N ha^{-1} could reduce yield of cerelia crops. Application of N fertilizer with high rate does not guarantee in improving crop yields (Abdurachman *et al. cit* Erythrina 2016; Daramy *et al*. 2017). The increase of grain yield is influenced by the increase of available N in soil. According to Singh *et al.* (1994), cowpeas absorb N in their biomass as much as 28-42 kg N ha^{-1} to produce grains of 0.45-0.85 Mg ha^{-1}, or uptake of 50 kg N ha^{-1} can produce grains of 1 Mg ha^{-1}. Cowpeas absorb N for protein formation in their tissues. Leave and grains of cowpea contain protein as much as 27-43% and 21-33%, respectively (Kyei-Boachen *et al*. 2017).

Table 3 also shows that residual N fertilizer in soil increases weight of biomass at harvest, although the biomass yields among residual N fertilizer treatments are not significantly different. The dry weight of biomass of cowpea cultivated in rainfed lowland ranged from 0.95 to 3.43 Mg ha^{-1}. Study of Rahmadani and Sunarlim (2013) also showed that variety had significant effect on dry weight of biomass in which dry weight of KT6 variety was 34% higher than KT7 and KT8 varieties. The biomass can be used as animal feed or returned back to the soil as green manure to improve soil fertility either physical, chemical, or biological.

Table 3. Yield of grains and dried biomass of three cowpea varieties applied with different amount of N fertilizer residue.

Variety	Residual N fertilizer rate (kg N ha^{-1})	Dry grain yield (kg ha^{-1})	Dry biomass weight (kg ha^{-1})
KT 9	0	1100 bc	1213 ab
	90	2330 a	3330 ab
	135	1683 ab	3430 a
	180	1603 bc	2243 ab
KT 6	0	923 c	953 b
	90	1693 ab	1537 ab
	135	1660 ab	2150 ab
	180	1747 ab	2880 ab
KT3	0	1260 bc	1030 ab
	90	1460 bc	1403 ab
	135	1580 bc	1897 ab
	180	1347 bc	1890 ab
Significance level		0.0215	0.2373
Coefficient of variance		24.62	22.03

The numbers followed by the same letters in the same column are not significantly different according to DMRT at 5% level.

CONCLUSIONS

The interaction between cowpea varieties and residual N fertilizer affected significantly emission of nitrous oxide (N_2O). Residual N fertilizer increases N_2O emission from cowpea cropping. Nitrous oxide emission from plots grown with cowpea variety of KT 9, KT 6, and KT 3 ranges 0.42-0.69, 0.30-2.64, and 0.09-2.19 kg N_2O ha^{-1}, respectively. Each cowpea variety has a diversity in releasing N_2O from soil. Variety of KT 9 with low N_2O flux has ability to utilize nitrogen from inorganic fertilizer more efficient compared to KT 6 and KT 3 varieties. Residual N fertilizer increases significantly grain yield of cowpea and available N content in soil. Residual N fertilizer increases available N in soil grown with KT 9, KT 6, and KT 3 with the range of 11.6-82.3%, 7.6-30.6%, 9.6-67.9%, respectively. Residual N fertilizer also increases significantly grain yield of KT 9, KT 6, and KT 3 varieties as much as 45.7-111.8%, 79.8-89.3%, and 6.9-25.4%, respectively.

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