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# Influence of Nitrification Inhibitor on Green House Gas (GHG) Emission under Intensive Paddy System

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**The field experiments were carried out to assess the influence of Dicyandiamide (DCD) along with nine different nitrogen management practices on emission of methane ($CH_4$) and nitrous oxide ($N_2O$) under intensive paddy system of Cauvery Delta Zone. The $CH_4$ and $N_2O$ efflux clearly showed daily and seasonal fluctuations. Among the treatments, Leaf Colour Chart (LCC) based N (30 kg N $ha^{-1}$ keeping the LCC value 4 as standard + DCD @ 10 % of applied N) recorded the lowest average emission of 0.50 $mg.m^2.day^{-1}$ and 2.93 $mg.m^2.day^{-1}$ of $N_2O$ and $CH_4$, respectively. Irrespective of all treatments, among the critical stages of crop growth, flowering stage recorded highest emission of 23 % $N_2O$ and 50 % of $CH_4$ when compared to tillering stage. Among the "N" applied treatments, Leaf Colour Chart (LCC) based N + DCD recorded the lowest seasonal green house gas emission (0.49 kg $ha^{-1}$ & 0.44 kg $ha^{-1}$ of $N_2O$ and 2.94 kg $ha^{-1}$ & 3.20 kg $ha^{-1}$ of $CH_4$ during Kharif and Rabi seasons, respectively) along with higher paddy yield (12 to 15%).**

**Key words:** Dicyandiamide, Nitrogen fertilizer, Nitrous oxide, Methane, Paddy

Methane and nitrous oxide are the two major Green House Gases (GHG) emitted from paddy (*Oryza sativa* L.) agro-ecosystem. At global level, rice cultivation alone contributes 10 % of the total $CH_4$ emission while the global warming potential of $N_2O$ is 298 times higher (Rees *et al.* 2013) than carbon di-oxide, so mitigation of both $CH_4$ and $N_2O$ is needed to combat global warming. In flooded rice, methanogens bacteria consume soil organic carbon and emit $CH_4$. In rice soil, $N_2O$ is produced by both biological (nitrification and denitrifcation) and chemical decomposition process. Nitrogen based fertilizer is main source of $N_2O$ production in rice soil. Methane and $N_2O$ production in rice soil is influenced by several factors such as water, soil pH, redox potential, temperature, organic matter of soil, soil microbial diversity, transplanting methods, rice cultivar, crop duration and type & time of fertilizer application (Hussain *et al.,* 2015). Application of "N" fertilizers increases $N_2O$ emissions (Bronson and Mosier, 1992). Emissions of $N_2O$ from N-fertilized croplands vary considerably, ranging between 0.001% and 6.8% of applied "N" (Bouwman, 1990; Eichner, 1990). From the agricultural soils, nitrification and denitrification are the two processes responsible for formation of $N_2O$. In both these processes, nitrite ($NO_2^-$) is formed as an intermediate compound. During the process of nitrification, $NH_4^+$, in aerobic condition, gets oxidized to $NO_3^-$ via hydroxylamine and nitrite, releasing $N_2O$ as a byproduct, while in denitrification, the $NO_3^-$ gets completely reduced to $N_2$ evolving $N_2O$ as an intermediate product. Therefore, the end product of nitrification works as substrate for denitrification. Hence, controlling the first process will certainly help in regulation of second process to ome extent. Nitrification inhibitors are compounds that reduce the rate at which ammonium is converted to nitrate either by killing or interfering with the metabolism of nitrifying bacteria. Dicyandiamide (DCD) is one of the most widely used bacterio-static nitrification inhibitors in the agriculture (Zacherl and Amberger, 1990) and decomposes in soil to non-toxic products. Effect of DCD on $N_2O$ emissions has been reported by Mosier *et al*. (1996) in wheat and maize and McTaggart *et al.* (1997) in ryegrass, grassland and spring barley. The present study was undertaken to observe the effect of DCD on $N_2O$ & $CH_4$ efflux from irrigated rice soils of Cauvery Delta Zone to assess its suitability for decreasing GHG emission to the atmosphere.

## Material and Methods

The present investigation was carried out during the year 2013-2014 to assess the influence of Dicyandiamide (DCD) along with nitrogen management practices on the emission of $N_2O$ & $CH_4$ from agricultural soils. A field experiment was conducted at Tamil Nadu Rice Research Institute, Aduthurai ($11^0$ N latitude, $79^0$ 31' E longitude, 19.4 MSL). During normal years, the annual rainfall is 1200 mm of which around 70 % is received during September to October (North East Monsoon). The climate of the experimental site (Cauvery Delta) is sub tropical monsoon type. The experiment with fixed plots has been laid out in a Randomized Block Design (RBD) with three replications. The details of the treatments are listed below.

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***Treatments***

$T_1$ : Absolute control

$T_2$ : Blanket recommendation of Nitrogen {150kg N $ha^{-1}$ in 4 splits 25 % each at basal, 15,30, and 45 Days After Transplanting (DAT)}

$T_3$ : Leaf Colour Chart (LCC) based N (30 kg N $ha^{-1}$ keeping the LCC value 4 as standard)

$T_4$ : SSNM (Site Specific Nutrient Management) based N with fixed split approach (35 % N at 15 DAT, 40 % N at 30 DAT, 25 % at 45 DAT with use of LCC at each stage)

$T_5$ : Early completion of N application (25 % basal 50 % at 20 DAT and 25 % at 40 DAT)

$T_6$ : $T_2$ + Dicyandiamide (DCD) @ 10 % of applied N

$T_7$ : $T_3$ + DCD @ 10 % of applied N

$T_8$ : $T_4$ + DCD @ 10 % of applied N

$T_9$ : $T_5$+ DCD @ 10 % of applied N

*Common application ($T_2$ to $T_9$): Each 50 kg $ha^{-1}$ of Phosphorus & potassium, Micronutrient mixture @ 25 kg $ha^{-1}$ and Gypsum @ 500 kg $ha^{-1}$

A uniform plot size of 25 $m^2$ was adopted for all the treatments and replications. Nitrogen was applied as per treatment schedule through urea while phosphorus and micronutrient mixture were applied entirely as basal and potassium in two equal splits (basal and at panicle initiation stage). The DCD was applied at the rate of 10% of applied N. Need based plant protection measures were taken up against pest and diseases.

***$N_2O$ and $CH_4$ measurements***

The $N_2O$ and $CH_4$ efflux from all the plots were measured using static chambers during the critical stages of crop growth (tillering, flowering and maturity). To collect the gas generated in the experimental plot, the acrylic chambers (60 cm height with the capacity of 80 lit) were placed on the iron base (47 cm diameter with the total area of 1562 $cm^2$) which was inserted 10 cm inside the soil one day before gas collection. The channel situated at the upper edge of base was filled with water to make the system air tight. One 3-way stopcock (Eastern Medikit Ltd. India) is fitted at the top of chamber to collect gas samples. The chamber should be thoroughly flushed several times with a 50 ml syringe to homogenize the inside air thoroughly. A battery operated fan was run continuously during the sampling to circulate the air inside the chamber to facilitate the proper mixing of the gas inside.

Gas samples were drawn with 50 ml syringes with the help of hypodermic needle (24 gauge) at 0, 10, 20 and 30 min. intervals and syringes were made airtight with a three way stop cock to arrest the gas diffusion. The soil temperature, chamber temperature and water level inside the iron base was recorded during gas collection which was used to calculate $N_2O$ & $CH_4$ flux. Air samples were brought immediately to the laboratory for $N_2O$ & $CH_4$ analysis. The $N_2O$ & $CH_4$ concentration was determined by using a gas chromatograph (Varian, 450 GC, German), equipped with Electron Capture Detector (ECD) and Flame Ionization *Detector* (FID), respectively. Under the appropriate operating conditions (column temperature 35°C, injection temperature 120°C and detection temperature 300°C) $N_2O$ & $CH_4$ peaks were detected at a specific retention time. Before sample analysis, gas chromatograph was calibrated with different dilutions of 10 ppm of $N_2O$ & $CH_4$ gas procured from M/S Multitech Pvt. Ltd., New Delhi, India.

**Results and Discussion**

The experimental soil is fine, montmorillonitic, isohyperthermic, Udorthentic Chromusterts under the soil textural class 'clay' coming under Kalathur soil series. It was neutral in pH with a soluble salt concentration of < 0.5 $dSm^{-1}$ (Table 1). The organic carbon status was medium falling in the range of 0.5 to 1 %. Owing to its heavy clay content, it possessed a high cation exchange capacity (42.3 C mol (p+) $kg^{-1}$).

**Table 1. Physico-chemical characteristics of experimental soil**

| Particulars | Values |
|---|---|
| Coarse sand (%) | 6.60 |
| Fine sand (%) | 26.27 |
| Silt (%) | 15.5 |
| Clay (%) | 47.0 |
| pH (1:2.5) | 7.77 |
| Electrical conductivity ($dSm^{-1}$) (1:2.5) | 0.32 |
| Organic carbon (%) | 0.66 |
| Cation Exchange Capacity (C mol (p+) $kg^{-1}$) | 42.3 |
| Available N (kg $ha^{-1}$) | 291 |
| Available P (kg $ha^{-1}$) | 66.0 |
| Available K (kg $ha^{-1}$) | 234 |

***Influence of different "N" management practices***

The measurement of $CH_4$ and $N_2O$ efflux was carried out from the soils amended with different nitrogen management practices along with 10 % of DCD. It showed daily and seasonal fluctuations. Among the treatments, Leaf Colour Chart (LCC) based N (30 kg N $ha^{-1}$ keeping the LCC value 4 as standard + DCD @ 10 % of applied N) ($T_7$) recorded the lowest average emission of 0.50 mg.$m^2$ $day^{-1}$ and 2.93 mg.$m^2$.$day^{-1}$ of $N_2O$ and $CH_4$, respectively (Fig. 1).

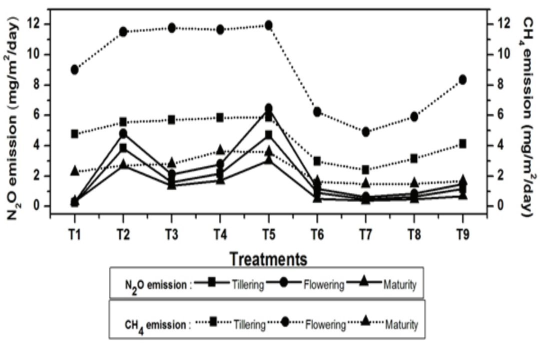


**Fig.1. Influence of different "N" management practices on $N_2O$ and $CH_4$ emission**

This might be due to the need based addition of urea under LCC guided N management along with DCD that have probably reduced the net $NO_3$-N available for denitrification in the soil and it also results in increased competition by plant roots. Irrespective of the days of fertilizer application, the maximum mean emission was recorded as 4.72 mg.m$^2$.day$^{-1}$ of $N_2O$ and 7.13 mg.m$^2$.day$^{-1}$ of $CH_4$ under early completion of N application (25 % basal 50 % at 20 DAT and 25 % at 40 DAT- $T_5$) due to higher available $NO_3$-N.

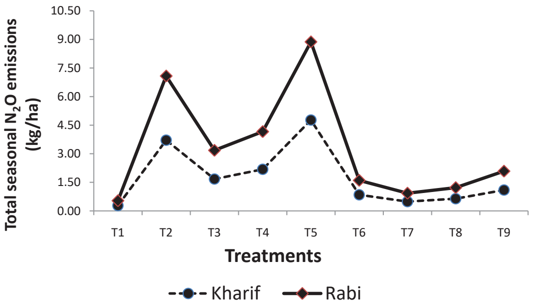


**Fig.2. Influence of different "N" management practices on $N_2O$ emission**

The peak $N_2O$ efflux observed during this study was associated with application of $NH_4$ based fertilizer, *i.e.* urea. Ammonium based fertilizer application directly stimulates nitrification process, as it serves the substrate for nitrifying bacteria in the oxic conditions. Nitrification inhibitor retards the ammonium mono-oxygenase enzyme which is responsible for converting ammonium present in soil to hydroxyl amine which is further oxidized to nitrite and then to nitrate (Prasad and Power 1995). Initial lower efflux at the start of the experiment was due to the time required for hydrolysis of urea in soil to $NH_4$. In agricultural fields, increase in $N_2O$ emission typically follows N-fertilization for a short time. After this, emission rates are reduced to fluctuate around a low base line level, independent of the amount of fertilizer applied (Mosier, 1998). A decline in the efflux is attributed to substrate depletion for nitrifying bacteria.

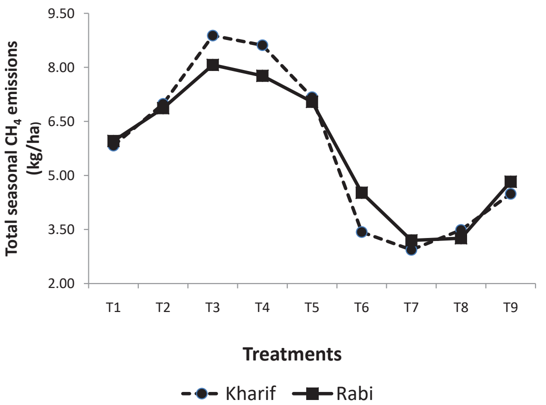


**Fig.3. Influence of different "N" management practices on $CH_4$ emission**

Irrespective of all treatments among the critical stages of crop growth, the flowering stage recorded higher emission of 23 % $N_2O$ and 50 % of $CH_4$ when compared to tillering stage (Fig. 1). This was due to the coincidence of top dressing of nitrogenous fertilizers and active growth stage of crop produced more plant hormones which supported methane emission.

Methane emissions were always lower in the presence than the absence of DCD from our experiments. The application of DCD with basal fertilizer could reduce $CH_4$ fluxes and hence significantly influenced $CH_4$ emission. Saturation of soil creates anaerobic conditions conductive for $CH_4$ emission, as methanogens are strict anaerobes. Therefore, lower inhibitive effect of DCD on $CH_4$ emission may be found when the DCD applied into soil with tillering and panicle initiation than with basal fertilizer in our experiments.

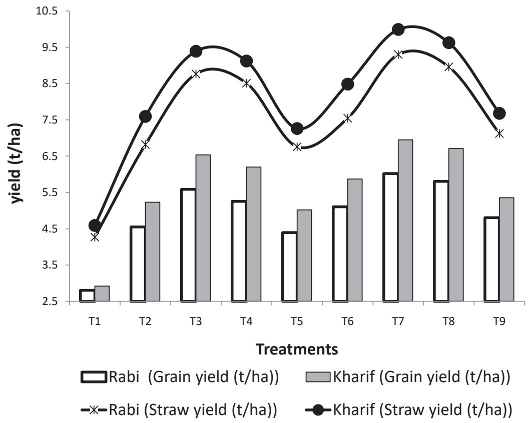


**Fig.4. Influence of different "N" management practices on paddy yield**

Application of fertilizers along with DCD has been considered to be one of potential mitigation strategies in rice paddy field due to the simultaneous reduction of $CH_4$ and $N_2O$. However, there are conflicting reports regarding the influence of urease and nitrification inhibitors on $CH_4$ emission (Malla *et al.,* 2005) and the mechanism of urease inhibitors and nitrification inhibitors on $CH_4$ emission needs to be considered from three different levels. Urease and nitrification inhibitors affected $CH_4$ emission by the influence on methanogenesis. Wang *et al.* (1991) indicated that urease inhibitors have little influence on the oxidation of ammonium following the hydrolysis of applied urea in soils, but hydroquinone may decrease $CH_4$ production via inhibition of the methanogenic fermentation of acetate.

Among the "N"applied treatments, Leaf Colour Chart (LCC) based N (30 kg N ha$^{-1}$ keeping the LCC value 4 as standard + DCD @ 10 % of applied N) ($T_7$) recorded the lowest GHG emission of 0.49 kg ha$^{-1}$ & 0.44 kg ha$^{-1}$ of $N_2O$ and 2.94 kg ha$^{-1}$ & 3.20 kg ha$^{-1}$ of $CH_4$ during Kharif and Rabi seasons, respectively. This might be due to the lower availability of substrate for $N_2O$ emission. The use of DCD reduced $N_2O$ emission significantly by 77-85% and $CH_4$ emission by 34-42 % relative to treatments without DCD. Besides increased "N' use efficiency & higher grain yield (6-7%) was observed in all DCD treatments. This is due to, firstly DCD reduces $N_2O$ emissions directly by nitrification (by reducing $NH_4^+$ to $NO_3^-$) as well as indirectly by de-nitrification (by reducing $NO_3^-$ availability in soil). Secondly, methane emission from rice soil can be reduced by enhancing methane oxidation and suppressing methane production and further by reducing the aerenchymal transportation through rice plant. Besides DCD maintained higher soil redox potential, this subsequently reduced the cumulative methane emission. Comparable results were also reported by Kumar and Malyan (2016).

The usage of DCD not only decreasing the GHG emission (30 to 80% reduction of $N_2O$ and $CH_4$) also increased the crop yield up to 12 to 15% in both kharif and Rabi. Irrespective of the seasons the treatment LCC based N +DCD recorded higher grain and straw yields (grain yield of 6.95 t $ha^{-1}$ during Kharif , 6.92 t $ha^{-1}$ during Rabi, straw yield was 9.99 t $ha^{-1}$ during Kharif and 9.30 t $ha^{-1}$ during Rabi) followed by SSNM based N+ DCD9G Fig. 4 & 5.).

This is due to the need based application of fertilizers in the treatments considerably increased the crop growth and yield. The DCD applied treatments registered higher grain and straw yields ranged from 5 to 22 % then others. This might be due to; DCD reduced the net $NO_3$-N available for denitrification in the soil as a result of increased competition by plant roots.

DCD is not a biocide and has no effect on soil microbial biomass (Di and Cameron, 2004). It acts specifically on an enzyme (ammonia monooxygenase) contained in nitrosomonas by blocking the site where ammonia is converted to nitrite. It is also water soluble and biodegradable into carbon dioxide, water and ammonia. Its rate of degradation and its effectiveness decreases with time after application to soils. Increasing the soil temperature, pH, moisture and organic matter content decreases its effectiveness (Irigoyen *et al.,* 2003; Di and Cameron, 2004). DCD is a commonly used nitrification inhibitor; several investigations had also proved that DCD worked as a potential nitrification inhibitor for Indian conditions (Majumdar and Mitra, 2004).

It is naturally broken down in the soil into non-toxic product with no traces of residue left beyond the cropping year. A reduction in the $N_2O$ to the tune of 40% in a dry sandy loam soils has been reported (Skiba *et al*., 1993), 58–78% when mixed with urea in grassland and barley fields (McTaggart *et al.,* 1997), 63% in lab condition (Pathak and Nedwell, 2001) and 52.6% in winter wheat when fertilized with urea (Bronson and Mosier, 1993).

## Conclusion

From the foregoing results of present study, it could be inferred that both LCC based N management along with 10% of DCD registered lower GHG emission. It is also reasonable to suggest that DCD have potential to decrease GHG emission and other wise increase the efficiency of N cycle. They are potentially additional tool to assist agriculture to achieve its economic and environmental goals considerably. Hence, as a simple tool, LCC based N management along with 10% of DCD is found to be the optimal N fertilization strategy for rice, since it gives lower GHG emission besides savings of N as compared to blanket N recommendation. More research is required therefore to move this emerging technology from the current "proof of concept" situation to a practical, cost effective technology on the farm

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