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Relationship between leaf nitrogen and chlorophyll content as influenced by nano urea spray in maize (*Zea mays* L.)

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Abstract
A field experiment to know the relationship between Leaf nitrogen and Chlorophyll content as nano urea application in Maize (*Zea mays* L.) was conducted during *Kharif* 2021 at the College of Agriculture, Navile, Shivamogga, on sandy loam soil. The experiment was laid out in RCBD with nine treatments replicated thrice. Treatments include combinations of basal application of prilled urea at 25 and 50% recommended N (RDN) with a spray application of nano urea at 15, 30, 45 and 60 DAS, which were compared with the package, absolute control and N management through LCC using nano urea. Before and ten days after the spray of nano urea developed a regression coefficient between leaf N and chlorophyll content at 15 DAS (R^2 = 0.48 & 0.59), 30 DAS (R^2 = 0.68 & 0.75), 45 DAS (R^2 = 0.61 & 0.80) and 60 DAS (R^2 = 0.78 & 0.87).

Keywords: Nano urea, prilled urea, leaf n content, leaf chlorophyll content

Introduction
Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice. It ranks fourth after rice, wheat and sorghum in India. It belongs to the Gramineae family. The nutritional value of maize is high and contains 72% starch, 10% protein, 8.5% fibre, 4.8% oil, 3.0% sugar and 1.7% ash (Hokmalipour *et al*., 2010) [5]. It is cultivated in the tropics, sub-tropics, temperate regions and semi-arid conditions. It occupies an area of 201.98 m ha globally, with a production of 1162.35 m t and a productivity of 5.75 t ha^{-1} (Anon., 2020) [1]. In India, it occupies an area of 9.89 m ha with a production of 31.64 m t and productivity of 31.99 q ha^{-1} (Anon, 2021) [2]. It is a C_4 plant which is exhaustive with high N requirements. N is the primary nutrient, a major constituent of proteins, nucleic acids, growth hormones, vitamins and chlorophyll. The leaf chlorophyll is influenced by the leaf N content and is essential for photosynthesis, affecting the growth and yield of maize. The application of external chemical fertilizers serves as a source of N for plants. Since green revolution, conventional fertilizer application has led to the loss of nutrients from agricultural fields through leaching and gaseous emissions, thereby causing environmental pollution. To enhance nutrient use efficiency with minimal threat to the environment, recently, nanotechnology with nano agri-inputs has emerged as an innovative solution (Kumar *et al*., 2021) [8]. The nano urea is one among the promising nano fertilizers claimed to be having N use efficiency of more than 80% as it contains nanoscale nitrogen particles (30-50 nm), with more surface area (10,000 times over 1 mm urea prill) and number of particles (55,000 nitrogen particles over 1 mm urea prill). Nano nitrogen particles with a pore size (20 nm) can easily penetrate through the cell wall and reach up to the plasma membrane. A 500 ml nano urea bottle contains four% N which is recommended to spray at rate of 4ml l^{-1} (Kumar *et al*., 2021) [8]. Thus, applying nano urea through foliar application leads to more efficient absorption and penetration of nitrogen, leading to better physiological growth and leaf health.

Material and Methods
A field experiment was conducted at College of Agriculture, Navile, Shivamogga during *Kharif* 2021. The experimental field was situated at 13° 58’ to 14°.1’ N latitude and 75° 34’ to 75°.42’ E longitude with an altitude of 615 meters above mean sea level and is located under the Southern Transition Zone of Karnataka. The soil was sandy loam in texture acidic in nature (pH 5.89) with a low salt load (EC 0.27 dS m^{-1}). It has a low status for organic carbon (4.63 g

kg^{-1}) and available nitrogen (226.72 kg ha^{-1}), while the status of available phosphorous (61.08 kg ha^{-1}) and available potassium (201.23 kg ha^{-1}) were high and medium, respectively. The experiment was laid out in RCBD with nine treatments replicated thrice. The total numbers of unit plots were 27 (9 treatment × 3 replication). Blocks were separated from each other by 1 m wide drains and the unit plots in each block by 30 cm bunds. The gross plot size was 6 m × 3.6 m and net plot size was 4.8 m × 2.4 m. Treatments were distributed randomly in the plots within the blocks. The test hybrid used was P - 3550 sown at a spacing of 60 X 30 cm. The interval and dose of prilled and nano urea application as per treatments is represented in Table 1.

Table 1: Interval and dose of prilled and nano urea application as per treatments in maize

Treatments details	Fertilizer application interval and dose (Bold number indicates nano urea spray)/ha						
	At sowing	15 DAS	30 DAS	45 DAS	60 DAS	Total normal N	Total Nano N
T_1: Absolute control	-	-	-	-	-	-	-
T_2: POP	75 kg	-	37.5 kg	37.5 kg	-	150 kg	-
T_3: 25% N basal through commercial N fertilizer + 3 sprays of nano urea at 30, 45 and 60 DAS	37.5 kg	-	52 g	60 g	70 g	37.5 kg	182 g
T_4: 25% N basal through commercial N fertilizer + 4 sprays of nano urea at 15, 30, 45 and 60 DAS	37.5 kg	43 g	52 g	60 g	70 g	37.5 kg	225 g
T_5: 50% N basal through commercial N fertilizer + 2 sprays of nano urea at 30 and 60 DAS	75 kg	-	52 g	-	70 g	75 kg	122 g
T_6: 50% N basal through commercial N fertilizer + 3 sprays of nano urea at 30,45 and 60 DAS	75 kg	-	52 g	60 g	70 g	75 kg	182 g
T_7: 50% N basal through commercial N fertilizer + 25% commercial N fertilizer as top dress at 30 DAS + 1 spray of nano urea at 60 DAS	75 kg	-	37.5 kg	-	70 g	112.5 kg	70 g
T_8: 4 sprays of nano urea at 15, 30, 45 and 60 DAS	-	43 g	52 g	60 g	70 g	-	225 g
T_9: N management through LCC (20% basal, remaining with nano N at 10 days interval)	30 kg	43g	47 g at 25 DAS; 55 g at 35 DAS	60 g	68 g at 55 DAS	30 kg	273 g

Note:
POP - 150: 75: 40 kg N: P_2O_5: K_2O ha^{-1}
DAS - Days after sowing
LCC - Leaf colour chart

For recording leaf chlorophyll and N content, randomly selected three plants were plucked from gross plots of various treatments before and ten days after spray of nano urea. From the same plants, three fully opened leaves were plucked for chlorophyll estimation and left over leaves of same plants were used for N content analysis and at 15, 25, 30, 40, 45, 55, 60 and 70 DAS.

Leaf chlorophyll analysis

Extraction of leaf chlorophyll was done using acetone extraction method (Arnon, 1949) [3]. Bottom and top portion of the leaves were discarded. The middle portion was cut into small pieces and 1.0 g of this cut leaf material homogenized with 10 ml of 80% acetone in a mortar with pestle. Homogenized solution was decanted and filtered the supernatant through a funnel using filter paper. Added sufficient quantity of 80% acetone and repeated the extraction. Finally, filtered volume made up to 25 ml in a volumetric flask. 0.2 ml of extract transferred into a test tube and diluted with 4 ml of 80% acetone.

Measure the absorbance at 645 nm, 652 nm and 663 nm against the solvent (80% acetone) blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following formula.

$$\text{Total chlorophyll content (mg g}^{-1}\text{ fresh weight}^{-1}) = \frac{27.8 \times \text{Absorbance at 652 nm} \times \text{Volume made}}{\text{Weight of sample} \times 1000}$$

$$\text{Chlorophyll a content (mg g}^{-1}\text{ fresh weight}^{-1}) = \frac{12.7 - A(652\text{ nm}) - 2.69 \times A(645\text{ nm}) \times \text{Volume made}}{\text{Weight of sample} \times 1000}$$

$$\text{Chlorophyll b content (mg g}^{-1}\text{ fresh weight}^{-1}) = \frac{22.9 - A(645\text{ nm}) - 4.68 \times A(663\text{ nm}) \times \text{Volume made}}{\text{Weight of sample} \times 1000}$$

Leaf N content analysis

The leftover leaves of three randomly plucked plants from gross plots of various treatments after using for chlorophyll estimation were shade dried and powdered by using mixer grinder with stainless steel blades. The powdered samples were stored in air tight containers for the analysis of leaf N.

N content in plant leaves was determined by Kjeldahl's method as described by Jackson (1973) [7]. Powdered samples were digested with concentrated sulphuric acid in the presence of digestion mixture (K_2SO_4: $CuSO_4.5H_2O$: Se powder in the ratio 100: 20: 1). The nitrogen content in the aliquot of digested sample was determined by distillation under alkaline medium. The liberated ammonia was trapped in boric acid mixed indicator and was titrated against standard sulphuric acid.

$$\text{Nitrogen (\%)} = \frac{\text{TV (ml)} \times \text{N.}H_2SO_4 \times \text{Dilution factor}}{\text{Weight of plant sample taken (g)}} \times 100$$

The relevant relationships like regression analysis were worked out from obtained data between leaf nitrogen and chlorophyll content, which were used for drawing out meaningful conclusions.

Results and Discussion

The leaf N and chlorophyll content normally established a relationship of 48% dependency of chlorophyll to that of leaf N content before spray of nano urea at 15 DAS (Fig. 1). Ten days after spray of nano urea (25 DAS) showed an improvement of 11% (Fig. 2) dependency indicating the effective uptake of N due to nano size and more surface area. Thereby, influenced to retain more chlorophyll content. Similar findings were observed by Benzon *et al.* (2015) [4] in rice.

Before spray of nano urea at 30 DAS showed a regression co efficient (R^2 = 0.68) between leaf N and chlorophyll content (Fig. 3). However, ten days after spray of nano urea indicated 10.29% increase in dependency between leaf N and chlorophyll content (Fig. 4) showing direct role of nano urea in improving the absorption of N applied with more efficiency. Before spray of nano urea at 45 DAS showed a regression co efficient (R^2 = 0.61) between leaf N and chlorophyll content (Fig. 5). Whereas, ten days after spray of nano urea developed a regression co efficient of 0.80 between leaf N and chlorophyll content (Fig. 6). Finally at 60 DAS before spray of nano urea developed a regression relationship of 78% (Fig. 7) dependency of leaf N and chlorophyll content it may be inferred that cumulative effect of spray of nano urea at 15, 30 and 45 DAS improved the dependency of 62.5% compared to before no nano spray to crop. There was regression co efficient of 0.87 obtained finally after ten days after spray of nano urea at 70 DAS (Fig. 8).

In general, it is noted that conjunction of basal and nano achieved a little higher value of chlorophyll constituents over without basal applications. The increase in chlorophyll a, b and total chlorophyll content was noticed because of direct and indirect role of N for increase in the formation of amino acids, RNA, DNA and energy compounds such as ATP as well as enzymes which lead to an increase in chlorophyll content. Nano N being nano sized particles depends on availability of leaf surface area at different days of growth for better absorption, permeability and penetration into plants leaves. A

Sufficient level of chlorophyll results in increased synthesis of the photosynthates essential for the plant's growth and development (Hopkins and Huner, 2004) [6]. According to Racuciu (2012) [10], nano nitrogen particles were able to penetrate the biological membranes of maize plant cells and increase chlorophyll pigments, by up to 38% when compared to the control. Van *et al.* (2013) [11] found that nanoparticles have a considerable impact on the chlorophyll concentration in Robusta coffee. The use of nanoparticles in the maize crop, particularly during the reproductive stage, enhanced the amount of chlorophyll in the leaves, according to Morteza *et al.* (2013) [9].

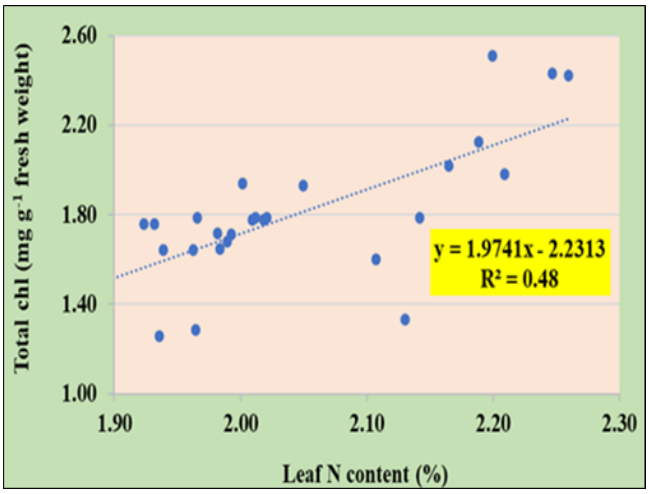

Fig 1: Regression relationship of total chl and leaf N content before spray (15 DAS) of nano urea in maiz

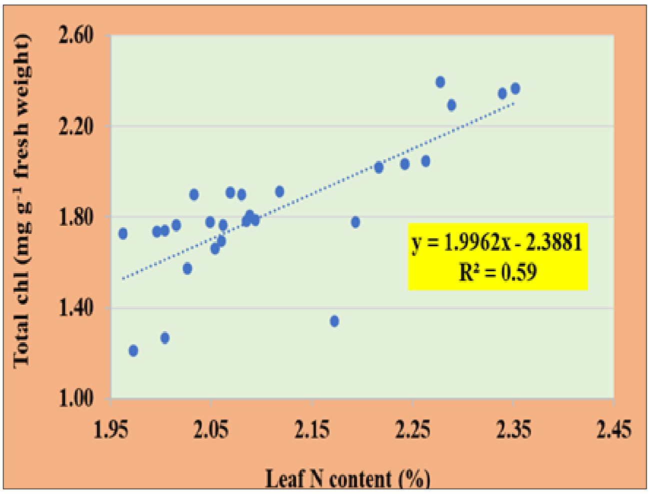

Fig 2: Regression relationship of total chl and leaf N content ten days after spray (25 DAS) of nano urea in maize

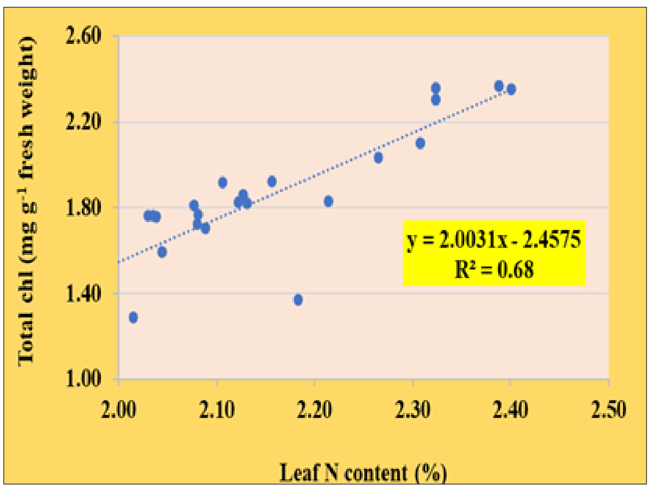

Fig 3: Regression relationship of total chl and leaf N content before spray (30 DAS) of nano urea in maize

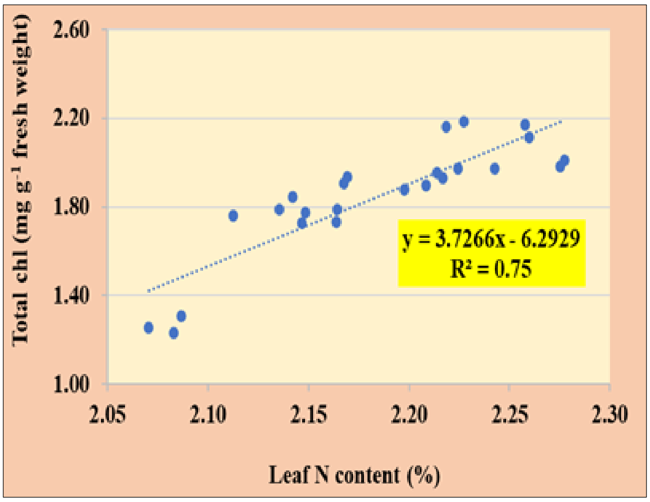

Fig 4: Regression relationship of total chl and leaf N content ten days after spray (40 DAS) of nano urea in maize

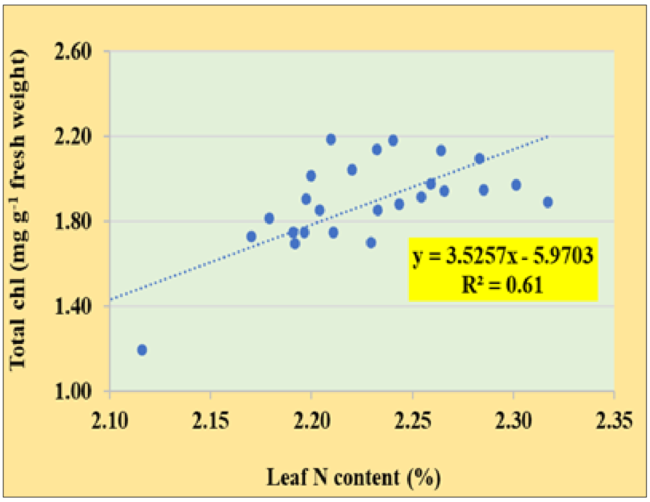

Fig 5: Regression relationship of total chl and leaf N content before spray (45 DAS) of nano urea in maize

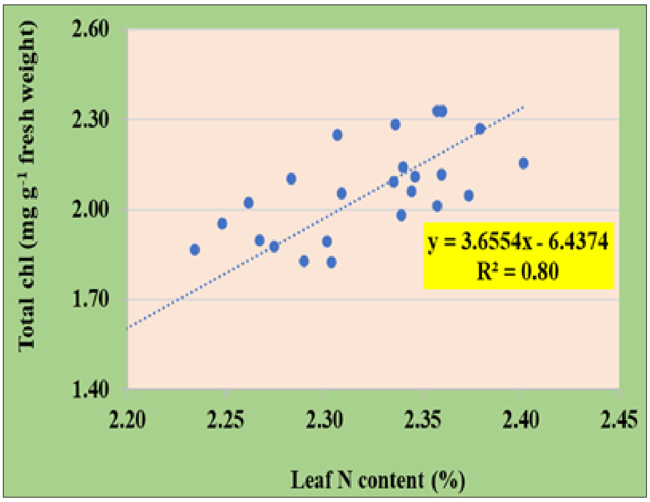

Fig 6: Regression relationship of total chl and leaf N content ten days after spray (55 DAS) of nano urea in maize

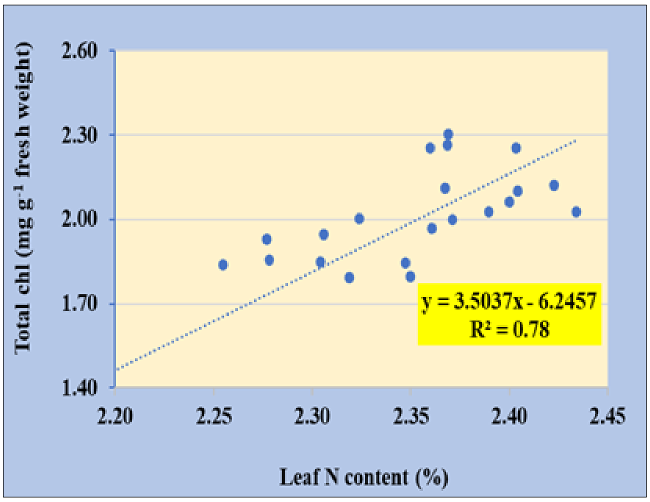

Fig 7: Regression relationship of total chl and leaf N content before spray (60 DAS) of nano urea in maize

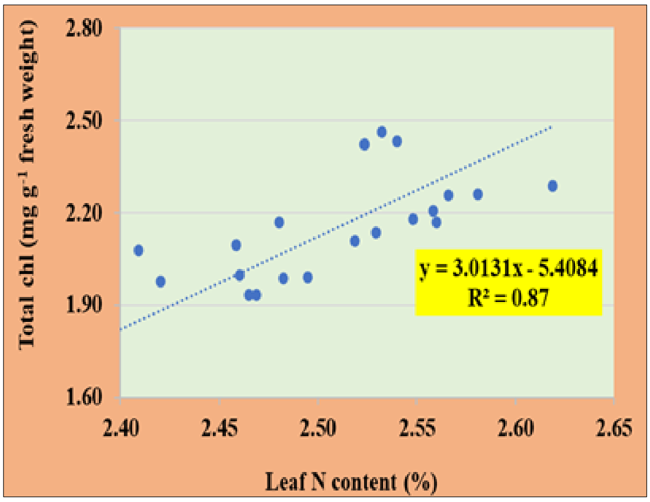

Fig 8: Regression relationship of total chl and leaf N content ten days after spray (70 DAS) of nano urea in maize

Conclusion

From the current investigation it was identified that nano urea spray application as shown positive relationship ($R^2 \geq 0.59$) between leaf N and chlorophyll with improvement in its content in maize leaf. The treatments with 50% application of conventional urea supported by nano urea spray as a replacement of top dressing of N fertilizers has shown a highly positive regression co efficient indicating the that basal application of conventional urea cannot be replaced by nano urea spray alone.

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