

**Evaluation of the effect of foliar nano nitrogen and zinc on chlorophyll (SPAD) and qualitative traits of green chilli in comparison with Urea and ZnSO<sub>4</sub>.**

**Abstract**

The faulty and imbalanced application of fertilizers to chilli threatens the environment and affects the country's sustainable food and nutritional security. Variation in the method and quantity of N and Zn fertilizer application has a higher impact on the chilli production and quality. An experiment was carried out at AHRS, Bavikere, KSNUAHS, Shivamogga, to study the effect of foliar nano nitrogen and zinc application on green chilli's growth, yield and quality. There were eight treatments which replicated three times using the RCBD design. Treatment combinations of foliar nano nitrogen and zinc and foliar urea and ZnSO<sub>4</sub> with different levels of RDN (75 %, 100 %, 125 %) were tested against control (100 % RDF). The results revealed that chilli quality parameters significantly varied with applying nano N and Zn compared to the control. Among the treatments, application of RDN (125 %) and foliar nano N (0.4 %) and Zn (0.4 %) at 30 and 45 DAT registered significantly higher chlorophyll, oleoresin, ascorbic acid and TSS. T<sub>3</sub> reported significantly superior ascorbic acid and oleoresin content to 14.94 per cent and 22.10 per cent over control.

**Keywords:** Green chilli, Nano fertilizers, Chlorophyll, Ascorbic acid, Oleoresin

**Introduction**

Chilli (*Capsicum annuum* L.) is an important commercial spice crop for small and marginal farmers of the world. India is the world's largest producer, user and exporter of chillies. In India, chilli is cultivated under 3,63,000 ha, producing 3783.21 metric tons. In India, Karnataka (673.81 metric t), Madhya Pradesh (669.55 metric t), and Andhra Pradesh (434.89 metric t) are the top contributing states (Anon., 2019). Indian chilli is considered famous for its colour and pungency levels.

In the Indian diet, chilli has a significant role as a condiment in many dishes. It is used as a spice and also in beverages and medicines. The pericarp and placenta of chilli contain an alkaloid 'capsaicin' responsible for pungency. Capsaicin is used to treat sensory nerve fibre disorder, diabetic neuropathy and in some ayurvedic treatments. Green chilli has high vitamin C (120 mg /100 g). One hundred grams of chilli carries 1.3 g of protein, 4.3 g of carbohydrate and 0.3 g of fat and 24 Kcal of energy. They are very high in potassium content and high in magnesium and iron. High vitamin C content can also significantly increase the uptake of non-heme iron from other constituents in a meal, for example, beans and grains. Vitamin C, also known as ascorbic acid, is an immune system stimulant that helps heal cellular damage

in the human body. Chilli has antibacterial action and is mentioned for treating worms in folk medicine. It helps reduce high blood pressure and lower the occurrence of heart attack and stroke. Oleoresin is the true core of spice and can substitute whole or ground spices without impairing their flavour and aroma (Shivaraman *et al.*, 2001).

Nitrogen is one of the fundamental macronutrients plants require for growth, development, and yield. It's also found in nucleic acids, coenzymes, and cell membranes, and it's involved in various metabolic processes such as cell division, photosynthesis, protein synthesis, plant growth and development, and shoot and root expansion. When nitrogen supply is low and application time is essential, the chilli crop is extremely sensitive to nitrogen (N) fertilizer application. Chilli plants require zinc for various biochemical activities, activating over 300 plant enzymes. Zn influences the actions of hydrogenase and carbonic anhydrase, ribosomal fractions' stability, and cytochrome production. Plant enzymes catalyzed by Zn are involved in glucose metabolism, cellular membrane integrity, protein synthesis, auxin synthesis regulation, and pollen development (Marschner, 1995). Zn is needed to control and maintain gene expression in plants that enable them to survive environmental stress factors (Cakmak, 2000). Zn is necessary to synthesize tryptophan, an IAA precursor, and the biosynthesis of auxin, a vital growth hormone. Traditional bulk fertilizers and conventional fertilizers may not only be costly for the producer but also potentially harmful to both people and the environment.

In the occurrence of nutrient deficiency, applying foliar fertilizer containing macro and micro nutrient elements can overcome the nutrient deficiency more quickly than fertilizer application to the soil. In foliar applications, fertilizers must cross the barrier the cuticle presents, following the lipophilic or the hydrophilic pathway. The lipophilic model involves diffusion through cuticular waxes, while the hydrophilic pathway is accomplished through polar aqueous pores obtainable in the cuticle and stomata. Since the diameter of cuticular pores has been projected around 2 nm, the stomatal pathway appears as the most likely route for penetration of nano fertilizers, with a size exclusion limit above 10 nm (Eichert *et al.*, 2008).

The search for smart fertilizers or ecologically friendly fertilizers—primarily those with high nutrient-use efficiency—has resulted from this, and nanotechnology is emerging as a possible substitute (Morab *et al.*, 2020). Nano fertilizers or nano-encapsulated nutrients have the properties to release nutrients effectively on-demand that regulate plant growth and enhance target activity. The nano-coated materials improve the penetration via stomata with a size exclusion limit above 10 nm (Perez, 2017). In addition, nano-carriers transport the nutrients in the right place and time and acts as the right source, reducing the extra amount of active chemicals deposited into the plant system and increasing nutrient use efficiency. Nano-fertilizers have a high surface area, sorption capacity, and controlled-release kinetics to targeted sites and have been considered the smart delivery system (Rameshaiah *et al.*, 2015). Thus, earlier workers reported significant responses by applying N and Zn through the foliar spray to chilli. However, the foliar application of nano nitrogen and zinc to chilli combined studies are insufficient or seldom nil. The present investigation studies the effect of foliar application of nano nitrogen and zinc on chilli quality parameters from the above facts.

## Materials and methods

Chilli (*Capsicum annuum* L.) cv. Arka Meghna, a hybrid chilli developed by IIHR, Bengaluru, was used in experimentation, which was conducted during the *summer* season in 2010-21 at Agricultural and Horticultural Research Station, Bavikere, KSNUAHS, Shivamogga, which lies on longitude and latitude of 75°51'E and 13°42'N, respectively at an altitude of 695 meters above the mean sea level. The experimental site falls under Karnataka's Agro-climatic Zone-VII (Southern Transition Zone - warm humid). Seedlings were raised in pro trays containing coir pith, soil and FYM. Temperatures during the experiment were 30-36 °C in the daytime and 21-15 °C at night. The relative humidity was 40 - 60 %, and plants were subjected to natural irradiance. One-month-old seedlings were transplanted in a randomized block design into the main field at a spacing of 75 cm × 45 cm.

The experiment consisted of 8 treatments, viz., Control (RDF) (T<sub>1</sub>), Control (RDF) with Two sprays of water (T<sub>2</sub>), RDN (125 %) with two sprays of nano N (0.4 %) and Zn (0.4 %) (T<sub>3</sub>), RDN (100 %) with two sprays of nano N (0.4 %) and Zn (0.4 %) (T<sub>4</sub>), RDN (75 %) with two sprays of nano N (0.4 %) and Zn (0.4 %) (T<sub>5</sub>), RDN (125 %) with two sprays of urea (2 %) fertilizer and ZnSO<sub>4</sub> (0.2 %) (T<sub>6</sub>), RDN (100 %) with two sprays of urea (2 %) fertilizer and ZnSO<sub>4</sub> (T<sub>7</sub>), RDN (75 %) with two sprays of urea (2 %) fertilizer and ZnSO<sub>4</sub> (T<sub>8</sub>), which replicated thrice.

The study was taken up on red sandy loam soil (pH 5.71, EC:0.24 dSm<sup>-1</sup>), low in organic carbon (0.56 %), low in nitrogen (215.64 kg ha<sup>-1</sup>), high in available phosphorus (50.56 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and high in available potassium (241.32 kg K<sub>2</sub>O ha<sup>-1</sup>). The DTPA extractable zinc was 2.32 mg kg<sup>-1</sup>.

Nanotechnology-based nano nitrogen and nano zinc (Liquid) fertilizers developed by IFFCO were used in this experiment. The nano N and nano Zn have nutrient concentration of 25000 and 5000 mg L<sup>-1</sup>. These fertilizers have a higher surface area (10,000 times over 1 mm fertilizer prill) and a number of particles (55,000 particles over 1 mm fertilizer prill). The average physical size of nanoparticles is in the range of 20 to 50 nm.

### Analysis of quality parameters

SPAD chlorophyll meter model was used for direct measurement of chlorophyll. The reading was taken at 45, 60 and 90 DAT.

The freshly harvested matured green chilli fruits were used for ascorbic acid estimation. Ten grams of fruit pulp was extracted with a four per cent oxalic acid solution and filtered. The filtrate was made up to 50 ml by using a four per cent oxalic acid solution. Five ml of fruit extract with 10 ml of oxalic acid (4 %) was titrated against 2, 6 dichlorophenol indophenol dye solution (V<sub>2</sub>). The endpoint was identified with the appearance of light pink colour. The working standard of ascorbic acid solution (100 µg ml<sup>-1</sup>) was also titrated in the same manner (V<sub>1</sub>). The amount of ascorbic acid was calculated using *equation 1* and expressed in mg 100 g<sup>-1</sup> of fruits (Sadasivam and Manickam, 1992).

$$\text{Ascorbic acid (mg 100 g}^{-1} \text{ sample)} = \frac{0.5 \text{ mg} \times V_2 \times 100 \text{ ml} \times 100}{V_2 \times 5 \text{ ml} \times \text{weight of sample (g)}} \dots \text{eq., 1.}$$

The total soluble sugar content of 5 fruits was measured by using a hand refractometer, and the mean observation of 5 plants was recorded from each treatment replication.

Oleoresin analysis was done by using the soxhlet apparatus. Chilli fruit was dried in a hot air oven at 50 °C. Two-gram fine chilli powder packed in filter paper and placed in a round bottom flask. The solvent acetone was used for the extraction. The solvent evaporated to dryness under a vacuum (AOAC, 1997). Percent oleoresin was calculated by using equation 2.

$$\text{Oleoresin (\%)} = \frac{\text{weight of oleoresin}}{\text{weight of sample}} \times 100 \dots \text{eq., 2}$$

## Results and Discussion

Data regarding chlorophyll content is presented in Table 1. Chlorophyll was measured using a SPAD meter at 45 and 60 DAT. Treatment that received RDN (125 %), foliar nano nitrogen, and zinc (64.74 and 67.03, respectively) recorded statistically significant chlorophyll content cover control. However, found to be similar to T<sub>4</sub> and T<sub>5</sub>. At 90 DAT, T<sub>3</sub> (73.47) was significantly superior to other treatments except for T<sub>4</sub>. The lowest SPAD observation was reported in T<sub>2</sub> (52.45, 57.80 and 60.24), where water spray was applied. The treatment T<sub>3</sub> reported a 14.27, 10.63 and 8.53 per cent increase in chlorophyll over control, respectively. It might be because foliar application of N increases leaf N and chlorophyll content, and chlorophyll and leaf photosynthetic rate are closely related. Applying K nanofertilizer obtained a higher biomass accumulation, nitrate reductase activity, photosynthetic activity, SPAD values and total chlorophyll content (Márquez-Prieto *et al.*, 2022). Upadhyaya *et al.* (2017) and Thunugunta *et al.* (2018) reported identical comments.

**Table 1: Effect of foliar nano nitrogen and zinc sources in comparison with conventional sources on chilli chlorophyll content (SPAD)**

Treatments	45 DAT	60 DAT	90 DAT
T <sub>1</sub> : Control (RDF) + RD Zn	52.45	58.67	61.25
T <sub>2</sub> : Control (RDF) + RD Zn + Two sprays of water	52.15	57.80	60.24
T <sub>3</sub> : RDN (125 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	64.74	67.03	73.47
T <sub>4</sub> : RDN (100 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	62.14	65.90	71.21
T <sub>5</sub> : RDN (75 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	60.25	64.67	68.15
T <sub>6</sub> : RDN (125 %) + Two sprays of 2 % urea fertilizer + Two	61.85	64.43	66.25

sprays of 0.2 % ZnSO <sub>4</sub>			
T <sub>7</sub> : RDN (100 %) + Two sprays of 2 % urea fertilizer + Two sprays of 0.2 % ZnSO <sub>4</sub>	57.75	62.63	65.16
T <sub>8</sub> : RDN (75 %) + Two sprays of 2 % urea fertilizer + Two sprays of 0.2 % ZnSO <sub>4</sub>	56.24	60.47	63.24
<b>S. Em ±</b>	0.95	1.16	1.27
<b>C D @ 5 %</b>	2.90	3.50	3.84

Note\*

Foliar applications were taken at 30 and 45 days after transplanting RD FYM and 100 % P<sub>2</sub>O<sub>5</sub> & K<sub>2</sub>O is common for all treatments  
 FYM- Farm Yard Manure, RDF- Recommended Dose of Fertilizer, RDN- Recommended Dose of Nitrogen  
 NS- Non significant

In order to face environmental challenges for survival, zinc may stimulate key enzymes involved in biochemical processes, such as pollen production, membrane integrity, glucose and protein growth regulator metabolism, and terminal oxidase in mitochondria. Nano Zn fertilizer may also enhance plant growth-promoting hormones (Verma *et al.*, 2022). Since nitrogen and zinc are involved in several metabolic pathways, their proportion in plants considerably impacts quality indices. Nutrient content in plant and quality parameters had a direct and positive correlation (Table 2). No significant difference was observed regarding TSS (Total soluble solids), but treatments that received nano fertilizers application had numerically higher TSS. It might be because the oxidative property of nano Zinc could have reduced soluble solids. Findings are relatable to Abdel Aziz *et al.* (2018) and Gulab *et al.* (2019). However, T<sub>3</sub> reported significantly superior ascorbic acid and oleoresin content to 14.94 per cent and 22.10 per cent over control, respectively. T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> were statistically at par with the best treatment, T<sub>3</sub>, regarding oleoresin content, but only T<sub>4</sub> was statistically similar to T<sub>3</sub> in ascorbic acid content (Table 2). Zinc fertilizers increased soluble carbohydrate concentration, probably due to the involvement of zinc in photosynthesis, chlorophyll synthesis, starch formation and enzyme carbonic anhydrase, accelerating carbohydrate formation, Wolska *et al.* (2018) and, in their findings, found similar results. Celsia and Mala (2014), Kisan *et al.* (2015), and other researchers reported significant improvement in quality parameters due to applying nano fertilizers.

**Table 2: Effect of foliar nano nitrogen and zinc sources in comparison with conventional sources on quality parameters of chilli**

Treatments	TSS (° brix)	Ascorbic acid (mg g <sup>-1</sup> )	Oleoresin content(%)
T <sub>1</sub> : Control (RDF) + RD Zn	7.22	154.92	14.25
T <sub>2</sub> : Control (RDF) + RD Zn + Two sprays of water	7.16	153.57	14.43

<b>T<sub>3</sub></b> : RDN (125 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	7.55	178.07	17.40
<b>T<sub>4</sub></b> : RDN (100 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	7.46	172.50	16.97
<b>T<sub>5</sub></b> : RDN (75 %) + Two sprays of 0.4 % nano N fertilizer + Two sprays of 0.4 % nano Zn	7.23	166.40	16.13
<b>T<sub>6</sub></b> : RDN (125 %) + Two sprays of 2 % urea fertilizer + Two sprays of 0.2 % ZnSO <sub>4</sub>	7.44	165.87	16.08
<b>T<sub>7</sub></b> : RDN (100 %) + Two sprays of 2 % urea fertilizer + Two sprays of 0.2 % ZnSO <sub>4</sub>	7.32	162.10	15.94
<b>T<sub>8</sub></b> : RDN (75 %) + Two sprays of 2 % urea fertilizer + Two sprays of 0.2 % ZnSO <sub>4</sub>	7.22	159.63	15.88
<b>S. Em ±</b>	0.13	2.37	0.44
<b>CD @ 5 %</b>	NS	7.19	1.33

Note\*

Foliar applications were taken at 30 and 45 days after transplanting

RD FYM and 100 % P<sub>2</sub>O<sub>5</sub> & K<sub>2</sub>O is common for all treatments

FYM- Farm Yard Manure, RDF- Recommended Dose of Fertilizer, RDN- Recommended Dose of Nitrogen

NS- Non significant

## Conclusion

Foliar feeding of both nano-N and Zn has significantly influenced the paddy growth and yield traits. Irrespective of sources of N, foliar application has improved quality parameters. Thus, the combined application of basal and foliar nano N and Zn should be considered for better quality. According to the results from experimental findings, foliar application of nano nitrogen and zinc in addition to RDF resulted in higher chlorophyll content, TSS, Ascorbic acid content and Oleoresin content.

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