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Influence of nano nitrogen and zinc sulphate on growth characteristics of lemon (*Citrus limon* L.)

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Abstract
The present investigation entitled "Influence of nano nitrogen and zinc sulphate on growth characteristics of lemon" was conducted at S. K. N. College of Agriculture, Jobner (Rajasthan) during 2021 on four years old uniform plants of lemon var. Pant Lemon-1. The experiment comprising of 16 treatment combinations with four levels of nano nitrogen (N_0-control, N_1 – nano nitrogen @ 1.50 ml/plant, N_2 – nano nitrogen @ 3.0 ml/plant and N_3 – nano nitrogen @ 4.50 ml/plant) and four levels of zinc sulphate (Z_0-control, Z_1- $ZnSO_4$ @ 0.40%, Z_2- $ZnSO_4$ @ 0.80% and Z_3- $ZnSO_4$ @ 1.20%) in Factorial Randomized Block Design with three replications. The application of treatment N_3 (Nano nitrogen @ 4.50 ml/plant) significantly increased growth characters *viz*., increment in plant height (27.64 cm), plant girth (4.96 cm), plant spread N-S (28.35 cm), plant spread E-W (30.02 cm), shoot growth (15.43 cm) and chlorophyll content (1.85 mg/g). Further, the foliar application of treatment Z_2 ($ZnSO_4$ @ 0.80%) significantly increased in growth parameters *viz*., increment in plant height (27.48 cm), plant girth (4.91 cm), plant spread N-S (28.31 cm), plant spread E-W (30.12 cm), shoot growth (15.33 cm) and chlorophyll content (1.83 mg/g).

Keywords: Nano nitrogen, plant, growth, girth and chlorophyll

Introduction
Lemon (*Citrus limon* L.) belongs to family Rutaceae, originated in South East Asia. It is commercially grown in tropical and subtropical region of India. Lemon is the fourth most important fruit after mandarin, sweet orange and acid lime and India ranks fifth among major lime producing countries (Anonymous, 2020) [3]. It is evergreen plant with dark green leaves; leaf lamina is lanceolate in shape having 70.2 mm in length and 30.2 mm in width with brevipetiolate attachment and denate margin. Leaf petiole is 8.6 mm in length and its wings are absent. Individual flowers are large, hermaphrodite and purple-tinged in the bud and on the lower surface of petals. Anthers are also yellow in color (Singh and Singh, 2006) [16]. Many flowers are staminate (Sterile male) because of pistil abortion, the incidence of which varies greatly from bloom to bloom and season to season (Reuther *et al*., 1967) [14]. Lemon plants bear flowers and fruits throughout the year proper manuring and fertilization has to be restored for obtaining highest yields and quality production which depends upon healthy and sturdy tree growth (Khehra and Bal, 2014) [9]. In North Indian condition, major bloom occurs during early spring (February-March). Fruits are acidic in nature with convex at base and rounded at apex having green-yellow skin color, 3 mm rind thickness, smooth surface and strongly conspicuous oil glands. Fruits having polyembryonic, smooth surface and creamy coloured seed with 0.5 in number per fruit (Singh and Singh, 2006) [16]. Citrus fruits and their by-products are of high economic and medicinal value because of their multipurpose uses, such as in food industry, cosmetics and folk medicine (Saidani *et al.*, 2004) [15]. There is also an increasing demand of "high quality fresh citrus" driven by World Health Organization recommendations. Citrus contain the largest number of carotenoids found in any fruit and an extensive array of secondary compounds with pivotal nutritional properties such as vitamin E, provitamin A, flavonoids, limonoids, polysaccharides, lignin, fiber, phenolic compounds, essential oils etc. (Iglesias *et al.*, 2007) [7]. It is also rich source of vitamin C (39mg/100g) and citric acid (5.41%) with a portion of protein (1%), carbohydrate (11.10%), Fat (0.90%), crude fiber (1.70%), Calcium (0.70%), phosphorus (0.01%) and iron (2.30mg/100g) (Attri and Maini, 1996) [5].

The growth and development of lemon tree are quite dependent on the nutrients applied at different stages of growth of both young and mature trees. Adequate supply of nitrogen, phosphorus and potassium is important for lemon growth. Nitrogen is key component in mineral fertilizers applied to citrus groves; it has more influence on tree growth, appearance and fruit production and quality than any other element. Phosphorus is necessary for many life processes such as photosynthesis and breakdown of carbohydrates and transfer of energy within the plant. Potassium is necessary for basic physiological function such as formation of sugars and starch, fruit development, synthesis of proteins and cell division and growth.

Micronutrients like zinc, iron and copper are not only essential but they are equally significant like other macronutrients, in spite of their requirement in minute quantities. They are the main elements responsible for the growth and development of plants. They also play a vital role in the various enzymatic activities and synthesis. Their acute deficiencies sometimes create a problem of incurable nature (Kumar, 2002) [10]. Nano-fertilizers are a new concept of nutrient management in crop and it is in its belonging stage there is a great thrust area in agriculture for sustainable crop improvement with major importance of nano-nitrogen. Nutrient release rate in nano-fertilizers can be controlled as per environment conditions like soil moisture percentage, temperature fluctuations and soil acidity level to effectively increase the efficiency of nutrients for plant growth compared conventional fertilizers. Nano-fertilizers are formulated to deliver and emit nutrient for more than 35 days deliberately and regularly. This may help in decreasing adverse effect on soil, plant and environment by enhancing the efficiency of applied nutrient and subsequently decrease leaching loss of nutrients (Naderi and Danesh-shahraki, 2013) [11]. Information regarding nano-N is meanly available very in case of horticultural crops.

Materials and Methods

The present investigation entitled "Influence of nano nitrogen and zinc sulphate on growth characteristics of lemon" was conducted at S. K. N. College of Agriculture, Jobner (Rajasthan) during 2021 on four years old uniform plants of lemon var. Pant Lemon-1. The experiment comprising of 16 treatment combinations with four levels of nano nitrogen (N_0-control, N_1 – nano nitrogen @ 1.50 ml/plant, N_2 – nano nitrogen @ 3.0 ml/plant and N_3 – nano nitrogen @ 4.50 ml/plant) and four levels of zinc sulphate (Z_0-control, Z_1-$ZnSO_4$ @ 0.40%, Z_2- $ZnSO_4$ @ 0.80% and Z_3- $ZnSO_4$ @ 1.20%) in Factorial Randomized Block Design with three replications. The nutrients were sprayed at two times. First spraying of nutrients was carried at full bloom stage in the second week of March and second spraying 30 days after first spray separately in the last week of April, during both the years. Spraying was done early in the morning. Each tree was sprayed heavily by taking care to wet the complete tree. It was fully ensured that all the sides of the tree were covered completely by the spray solution.

Plant height increment (cm)

The height of each experimental tree was measured with the help of a graduated staff from the bottom to the top of the tree at the beginning and at the end of growing season and expressed as increment in tree height in centimetres (cm).

Increase in plant height (cm) = (P_2-P_1)
P_2- Plant height at harvest
P_1- Plant height at the time of treatment application

Plant girth increment (cm)

The trunk girth of each experimental tree was measured 10 cm above the graft union at the beginning and end of the growing season and expressed as increment in trunk girth in centimetres (cm).

Increase in trunk girth (cm) = (P_2-P_1)
P_2- Trunk girth at harvest
P_1- Trunk girth at the time of treatment application

Plant spread increment [E-W and N-S (cm)]

The spread of the plant canopy in East–West and North–South direction was measured by using a measuring tape and was expressed in centimetre (cm). The initial observations were taken before the incorporation of the treatments and the final observations were taken after the harvesting of fruits. The increment in the canopy spread was calculated by subtracting the initial value from the final value and expressed in centimetres (cm).

Increase in plant spread (cm) = (C_2-C_1)
C_2= Plant spread at harvest
C_1= Plant spread at time of treatment application

Shoot extension growth (cm)

Five shoots were randomly selected from all over the periphery of each experimental tree and their length was measured with a measuring tape at the end of growing season and expressed in centimetres (cm).

Total Chlorophyll content (mg/g)

Total chlorophyll content of fresh leaf samples at flowering was determined by using DMSO. A known weight of sample (0.1 g) was taken in attest tube containing 10 ml DMSO solution and then the samples were kept in an oven for about 45 minutes at 40 °C for the extraction of chlorophyll pigments and allowed to cool in room temperature. The absorbance of the sample was measured at 645 nm and 663 nm wavelength filter in uv-vis spectrophotometer against a blank (100%) DMSO. The following formula (Aron, 1949) [4] was used for determination of total chlorophyll and expressed as mg/g of tissues.

The amount of chlorophyll present in the extract was calculated using the following equations:

$$\text{Total Chlorophyll mg/g tissue} = \frac{20.2\,(A645) + 8.02\,(A663) \times V}{1000 \times W}$$

A = Absorbance at specific wave length
V = Final volume of chlorophyll extract
W = Fresh weight of tissue extracted

Results and Discussion

Plant height

The data presented in table 1 revealed that foliar application of nano nitrogen significantly influenced the plant height increment in lemon. The maximum plant height increment (27.64 cm) was observed in treatment N_3 (Nano nitrogen @ 4.50 ml/plant) which is significantly superior than the rest of the treatments except treatment N_2 and minimum (21.42 cm) found under control. Further, data showed that application of zinc sulphate also significantly increments in plant height

during experimentation. The highest increment in plant height (27.48 cm) was registered under treatment Z_2 ($ZnSO_4$ @ 0.80%) whereas minimum (21.61 cm) in treatment Z_0 (control). The maximum amount of nano nitrogen and zinc stimulates the production of auxins that encourages cell division and cell elongation of plant and consequently resulted in increased plant height and other growth attributing characters (Al-gym and Al-asady, 2020) [2]. Similar results were reported by Benzon *et al.* (2015) [6] and Jhanzab *et al.* (2015) [8].

Plant girth

Data pertaining to the effect of nano nitrogen are presented in table. The foliar application of various nano nitrogen levels showed significant effects on increment in plant girth. The maximum increment in girth (4.96 cm) was observed in treatment N_3 (Nano nitrogen @ 4.50 ml/plant) followed by treatment N_2 (Nano nitrogen @ 3.0 ml/plant) and minimum (3.48 cm) under control. Therefore, the foliar spraying of treatment Z_2 ($ZnSO_4$ @ 0.80%) exhibited maximum increment in girth (4.91 cm) whereas minimum (3.61 cm) under control. The results indicated that treatment Z_2 found statistically at par with Z_3 ($ZnSO_4$ @ 1.20%) and significant over rest of the treatments.

Plant spread

The maximum increment in spread N-S (28.35 cm) was recorded with foliar application of treatment N_3 (Nano nitrogen @ 4.50 ml/plant) which was found statistically at par with treatment N_2 (Nano nitrogen @ 3.0 ml/plant). However, minimum increment in plant spread (20.52 cm) noted under control. Further, foliar application of zinc sulphate @ 0.80% was recorded maximum (28.31 cm) increment in plant spread followed by zinc sulphate @ 1.20% and minimum (21.26 cm) under control.

It is evident from data that foliar application of various nano nitrogen levels showed significant effect on increment in plant spread (E-W). The maximum increment in spread (30.02 cm) was recorded with application of treatment N_3 (Nano nitrogen @ 4.50 ml/plant) which was found statistically at par with treatment N_2 (Nano nitrogen @ 3.0 ml/plant). However, minimum increment in spread (22.54 cm) noted under control. Application of zinc sulphate significantly increased the plant spread (E-W) during experimentation. The application of treatment Z_2 ($ZnSO_4$ @ 0.80%) exhibited maximum increment in spread (30.12 cm) whereas minimum (22.92 cm) under control. The results indicated that treatment Z_2 found statistically at par with Z_3 ($ZnSO_4$ @ 1.20%) and significant over rest of the treatments. Nano-nitrogen and zinc provide more space for various metabolic reactions in the plant that increase the rate of photosynthesis, causing an increase in dry matter production, carbohydrates and also improvement in vegetative growth of plant (Qureshi *et al.*, 2018) [12].

Shoot growth

The foliar application of different levels of nano nitrogen significantly increases the shoot growth. The maximum shoot growth (15.43 cm) was found in treatment N_3 (Nano nitrogen @ 4.50 ml/plant) which was statistically at par with treatment N_2. However, minimum shoot growth (11.54 cm) observed under control. Further, the data revealed that maximum shoot growth (15.33 cm) was observed under treatment Z_2 ($ZnSO_4$ @ 0.80%) whereas, the minimum (11.89 cm) under control. However, treatment Z_3 ($ZnSO_4$ @ 1.20%) registered statistically at par with Z_2. The beneficial effect of nitrogen in increasing the plant growth might be due to the fact that absorbed nitrogen combined with carbohydrates synthesis leads to the formation of nitrogenous compound such as protein, nucleic acids, nucleotides, enzymes and co-enzymes to build up new tissues (Rathore and Chandra, 2003) [13].

Total Chlorophyll content

Foliar application of nano nitrogen had significant effect on chlorophyll content in leaves of lemon. It is apparent from the data that maximum and significantly better chlorophyll content was recorded when nano nitrogen was foliar applied @ 4.50 ml/plant as compared to other treatments. However, application of nano nitrogen @ 3.0 ml/plant was found statistically at par to nano nitrogen @ 4.50 ml/plant during the experimentation. A further reference to data showed that foliar application of zinc sulphate @ 0.80% also significantly increased the chlorophyll content in leaves of lemon during experiment over rest of the treatments except zinc sulphate @ 1.20% which was remained at par to it. The maximum chlorophyll content in leaves of lemon (1.83 mg/g) was recorded with application of zinc sulphate @ 0.80%. The incensement of chlorophyll due to the role of nano particle in improvement of leaves photosynthesis and decreasing the respiration rate (Abdel *et al.*, 2019) [2].

Table 1: Influence of nano nitrogen and zinc sulphate on growth characters

Treatments	Plant height increment (cm)	Plant girth increment (cm)	Plant spread increment N-S (cm)	Plant spread increment E-W (cm)	Shoot growth (cm)	Chlorophyll content (mg/g)
Nano nitrogen levels						
N_0 (Control)	21.42	3.48	20.52	22.54	11.54	1.50
N_1 (1.50 ml/plant)	25.16	4.32	25.81	27.46	13.78	1.69
N_2 (3.0 ml/plant)	27.45	4.87	28.18	29.89	15.30	1.82
N_3 (4.50 ml/plant)	27.64	4.96	28.35	30.02	15.43	1.85
SEm±	0.66	0.12	0.68	0.69	0.44	0.02
CD (P=0.05)	1.90	0.33	1.97	1.98	1.27	0.06
$ZnSO_4$ Levels						
Z_0 (Control)	21.61	3.61	21.26	22.92	11.89	1.52
Z_1 (0.40%)	25.32	4.29	25.31	27.09	13.74	1.71
Z_2 (0.80%)	27.48	4.91	28.31	30.12	15.33	1.83
Z_3 (1.20%)	27.26	4.81	27.98	29.78	15.10	1.80
SEm±	0.66	0.12	0.68	0.69	0.44	0.02
CD (P=0.05)	1.90	0.33	1.97	1.98	1.27	0.06
CV (%)	8.94	9.07	9.21	8.66	10.84	4.40

Conclusion

On the basis of results of present study, it was concluded that foliar application of nano nitrogen at the rate 4.50 ml/plant and zinc sulphate at the rate 0.80%) was found to be the best for maximum increase in plant height, girth, plant spread, shoot growth and chlorophyll content.

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