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Effect of Zinc Oxide Nanoparticles on Growth and Germination of Mung Bean Seeds (*Vigna radiata* L.)

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The globe is currently experiencing a micronutrient shortage, which is being addressed with various chemical fertilizers that harm the environment and human health. Research is shifting toward nanotechnology to overcome this and using nano fertilizer has sparked scientists' interest. In this study, we examined the effect of zinc oxide nanoparticles (ZnO NPs) dosage on the growth and seed germination of mu ngbean (*Vigna radiata* L.). This experiment was designed to study the impact of ZnO NPs, obtained from *Curcuma longa* leaves extract, on seedling vigour index, root, shoot length and chlorophyll content for *Vigna radiata* species. Three concentrations (3, 6 and 9 mg/mL) of ZnO NPs were examined at the seed germination stage. Significant enhancement of the germination percentage values was observed after the treatment of mungbean seeds with ZnO NPs in comparison with untreated seeds. Green-produced ZnO nanoparticles positively impacted the development and germination of *Vigna radiata,* indicating a beneficial impact on agricultural productivity. The response to ZnO NPs was dose-dependent, though, therefore more research is needed to determine the ideal dosages for increased agricultural production and human nutrition.

Keywords: Nanoparticles, Zinc oxide, *Vigna radiata*, *Curcuma longa*, Nanofertilizer.

INTRODUCTION

India stands second in agriculture production worldwide [1]. Haryana state is one of the biggest contributors to India's pool of food grain and is known as the food bowl of India. Though Haryana stands second in grain production, at the same time is dealing with the issue of diminishing size of farm holding, cultivable region, imbalanced utilization of manures, harsh environment and miniature supplement (micro-nutrient) deficiency in a plant. Micro and macronutrients are fundamental parts of the development of plant [2] and their deficiency is a danger to nourishment esteem, horticulture manageability and worldwide food security. A large number of soil samples were evaluated using GPS to find out micronutrient deficiencies in India and revealed the soil deficiencies as 36.5% for zinc, 23.2% for boron, 7.1% for manganese, 4.2% for copper and 12.8% for iron [3,4]. Similarly in Haryana, 5673 soil tests were investigated and reported 15.3% zinc deficient, 3.3% boron deficient, 6.1% manganese deficient, 5.2% copper-deficient, 21.6% iron-deficient soil. Pulses/cereal grains/seeds are the primary source of daily calorie consumption; however, they have a very low level of zinc and growing them on zinc-deficient soils reduces even more zinc concentration. Inadequate consumption of zinc-deficient foods can induce malnutrition syndrome. According to Pathak *et al.* [5], 73.5% of pregnant women in Faridabad city in Haryana had low zinc levels. According to Nidumuru *et al.* [6], zinc deficiency decreases the immunity of individuals and leads to thalassemia.

The use of chemical fertilizers is a solution to micro-nutrient deficit soil and also improves agricultural productivity however; the usage of chemical fertilizer is hazardous and not only causes contamination but also reduces soil quality. Due to their exceptional physical and chemical characteristics and their capacity to improve plant health, nanotechnology offers the greatest solution to this problem [7]. Along with this, they can increase crop yields by accelerating seed germination, seedling development and photosynthetic activity [8,9]. As they are more soluble and reactive, they can boost penetration through the cuticle, allowing for targeted distribution and controlled release of nutrients [10]. Nano-fertilizers serve as essential nutrients that extend beyond conventional fertilization, as they not only enhance crop growth but also contribute to restoring the environment to its natural equilibrium. Moreover, they have an advantage of being able to be administered in small amounts [11].

Among all metal oxide nanoparticles, zinc oxide is considered one of the most highly efficient. Zinc is a trace metal, which is involved in cell metabolism, growth, protein synthesis and proliferation. Of its broadband gap and strong excitonic binding energy, zinc oxide is important for both industry and research [12]. ZnO NPs also exhibit excellent optical, physical and anti-bacterial characteristics, making them ideal for agricultural applications. Nanoparticles of ZnO are biodegradable material that has photo-oxidizing and photocatalytic effects on the biological and chemical species [13,14]. There are over 300 enzymes that require zinc to function. One of the important pulses of India is mung bean and India produces 1.5 to 2.0 billion tonnes of Mung annually on the 3 to 4 million acres of land, with an aggregate capacity of 500 Kg/ha. The yield of green gram constitutes approximately 10-12% of total production in the country; nevertheless, nearly half of the soils utilized for food crop cultivation are deficient in plant-available zinc, leading to diminished crop productivity and nutritional quality of the harvested grains. Minimal study has been conducted on the impact of ZnO NPs on crops, mung bean, which is a frequent basic diet for most of the people. This study aimed to assess the impact of varying dosages of biogenic ZnO nanoparticles on seed germination and seedling characteristics in mung beans (V*igna radiata*).

EXPERIMENTAL

Synthesis of zinc oxide nanoparticles using leaf extract of *Curcuma longa*: ZnO nanoparticles were prepared by biological methods according to Singh *et al.* [15]. The leaves of *C. longa* were collected from the local agricultural land in Rohtak, India. Following an extensive wash with distilled water to eliminate any contaminants, the moisture was extracted by air-drying the leaves in shade, and the leaf powder was subsequently made using a grinder. For the synthesis of ZnO NPs, 10 g of leave powder was soaked in double distilled water (100 mL), stirred for 1 h using a magnetic stirrer at 60 ºC, filtered with Whatman no. 1 filter paper and finally the extract was refrigerated [16]. In the next step, in 80 mL of double distilled water, 0.02 M of $Zn(CH_3COO)\cdot 2H_2O$ and 20 mL of leaf extract was mixed followed by 2 M NaOH solution, resulting in the formation of white solution with a pH of 12. The solution undergo magnetic agitation and was reheated until it transformed into a pale yellow paste that eventually became creamy white. This product was subsequently extracted and rinsed multiple times with double distilled water, followed by a final rinse with ethanol to eliminate impurities from the final products. The solution was collected and dried in an oven overnight at 60 ºC. An obtained creamy white powder was carefully collected and preserved for characterization.

Characterization: The ZnO NPs were first characterized using UV-visible spectroscopy using a spectrophotometer in the 250–600 nm wavelength range. FESEM was used to determine the size and shape of the ZnO NPs using Jeol JSM-7800F field emission scanning electron microscope with 0.7 nm resolution. The FTIR spectra were recorded at a resolution of 4000-500 cm^{-1} using by Perkin-Elmer Spectrum 1000 spectrum in attenuated total reflection mode.

Seeds: *Vigna radiata* variety SML 668 was procured from the local seed shop at Rohtak city, India. Healthy seeds were chosen to reduce germination errors. The seeds were surface sterilized with 0.2% $HgCl_2$ and rinsed with distilled water.

Treatment studies: $ZnSO_4$ as a standard zinc source used by farmers due to insoluble bulk ZnO powder was also taken. $ZnSO_4$ and biosynthesized ZnO nanoparticles were suspended in double distilled water and dispersed using an ultrasonicator. Six distinct doses of aqueous $ZnSO_4$ (1 mM (4 mg), 2.1 mM (8 mg) and 4 mM (12 mg), as well as 3 mg (1.2 mM), 6 mg (4 mM) and 9 mg (7.1 mM) of biosynthesized ZnO NPs, were used for the seed treatments. The sterilized seedlings were immersed in their corresponding concentration for 2 h. The treated seedlings were placed on petri plates with Whatman No. 1 filter paper and 2 mL of water added. Petri plates were covered and stored in a dark environment. For 7 days, shoot and root lengths were measured every other day and the prepared solution was given in 5 mL concentration to their respective petri dish.

Seedling vigour index (SVI): The seedling vigour index (SVI) was calculated by using the following formula suggested by Abdul-Baki & Anderson [17]:

$$I = [\text{Germination (\%)}] \times [\text{Root length (cm)} + \text{Shoot length (cm)}]$$

$$\text{Relative seed germination rate (RSG)} = \frac{\text{Seeds in control which are germinated (SC)}}{\text{Seed in sample which are germinated (SS)}} \times 100$$

$$\text{Relative root growth (RRG)} = \frac{\text{Root length in sample (RS)}}{\text{Root length in control (RC)}} \times 100$$

Total chlorophyll: Total chlorophyll was determined according to Arnon *et al.* method [18], In brief, 25 mg of fresh leaf from each petri dish containing $ZnSO_4$ and ZnO NPs was manually blended with 10 mL of 80% acetone, and absorbance was measured at 645 and 663 nm using a spectrophotometer.

To calculate the chlorophyll content, the following formulae was utilized:

$$\text{Chlorophyll a (mg/g)} = 12.7\ (A663 - 2.69(A645) \times V\ 1000 \times W$$

$$\text{Chlorophyll b (mg/g)} = 22.9\ (A645) - 4.68(A663) \times V\ 1000 \times W$$

$$\text{Total chlorophyll} = 20.9 \times (A645) - 8.02 \times (A663) \times V\ 1000 \times W$$

where A = absorbance, V = final volume of sample, W = fresh weight of sample taken.

Statistical evaluation: A randomized block design with three replications was used to organize the treatments. ANOVA was employed to do a statistical analysis of the data.

RESULTS AND DISCUSSION

In this work, ZnO NPs was synthesized using *Curcuma longa* leaf extract which acts as both surface stabilizing and reducing agents. Zn^{2+} ions from zinc precursor *i.e.* zinc acetate dihydrate reacted with reducing agents, such as leaf extract and NaOH, to obtain ZnO nanoparticles. The visual interpretation revealed a change from pale yellow to white, indicative of nanoparticle formation, which were subsequently dried and preserved as a powder.

UV-Visible studies: The UV-Vis absorption spectra of the biosynthesized ZnO nanoparticles is shown in Fig. 1. An absorption peak at 380 nm was detected, which can be attributed to the inherent band gap of ZnO nanoparticles. The findings were quite similar to the reported investigations [19,20].

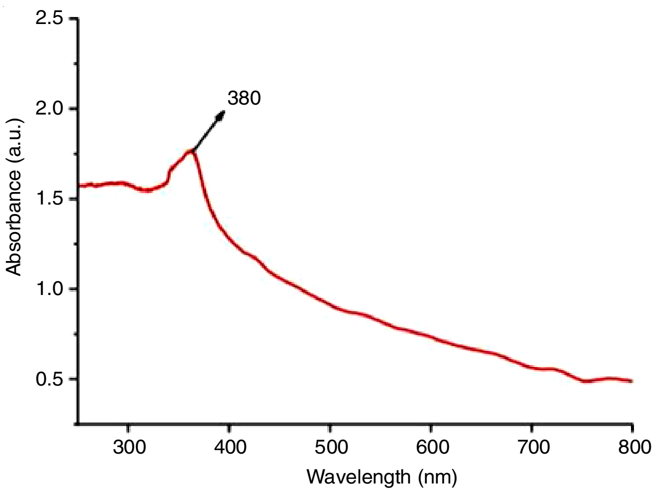

Fig. 1. UV-visible spectra of ZnO NPs synthesized using leaves extract of *Curcuma longa*

Morphological studies: The FESEM image of biosynthesized synthesized ZnO nanoparticles with different magnifications is shown in Fig. 2. The irregular to almost distinctive and nearly spherical morphology of nanoparticles were observed. The morphology and size can be influenced by various bioactive agents like polyphenols and other compounds. The results were quite similar to Alrajhi & Ahmed [21].

FT-IR studies: Fig. 3 shows the several functional groups present in ZnO nanoparticles which was synthesized with turmeric leaves. In turmeric, the ZnO peak at 875.68 cm^{-1} describes the stretching of C-H bend with sp^2 hybridization and the presence of *cis*-distributed alkene group. The other small wide peaks were observed at 1386.81 cm^{-1} and 1527.62

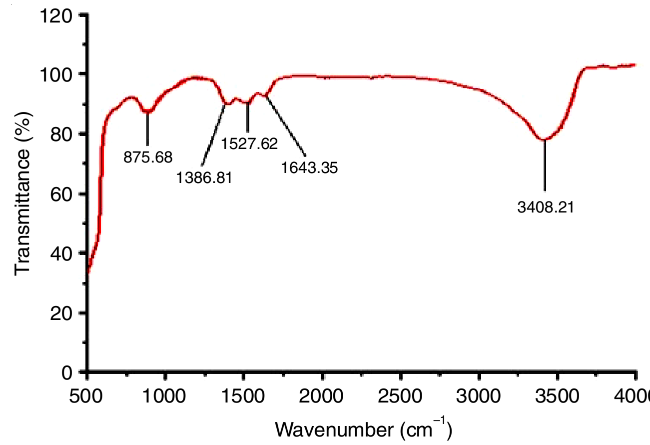

Fig. 3. FTIR spectra of synthesized ZnO NPs using leaves extract of *Curcuma longa*

cm^{-1} which shows the medium symmetric nitro group and strong asymmetric nitro group. A small broad peak has also been appeared at 1643.35 cm^{-1}, indicating C-C stretching with sp^2 hybridization and the presence of a saturated amide group. A broad peak was identified in the turmeric based ZnO NPs spectra at 3408.21 cm^{-1}, indicating the presence of significant amides with the primary NH_2 stretching and weak amines with secondary NH_2 stretching. These FTIR results are closely resemble those of a recent investigation by Jayarambabu *et al.* [22].

Evaluation of germination and growth parameters using biosynthesized ZnO nanoparticle treatments: ZnO NPs as well as $ZnSO_4$ salt had a significant impact on the seed germination. The higher seedling development and the maximal germination were observed in the seeds treated with lower concentrations of nanoparticles. Maximum germination and the highest seedling growth were displayed by the seeds treated with a lower quantity of nanoparticles. Similar findings in low concentrations of nanoparticles demonstrated stimulatory action and *vice versa*, were reported by Singh *et al.* [15]. The capacity of nanoparticles to pierce plant seed coverings and improve the seed germination and growth is thought to be responsible for the increase in germination. The impact of ZnO NPs varies depending on the species and concentration size. The shoot and root growth patterns show a huge variation dependent on dose as shown in Fig. 4, the zinc supplement which was supplied

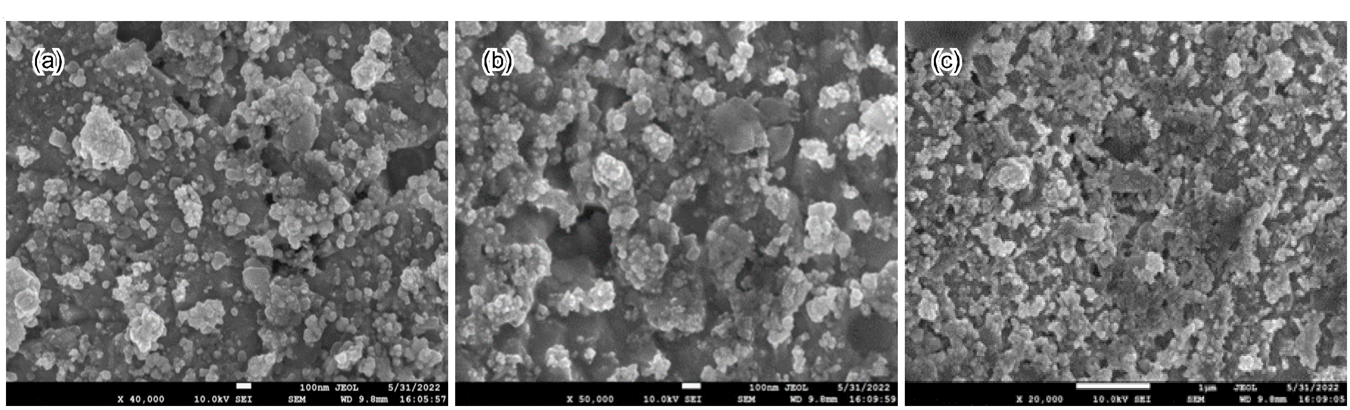

Fig. 2. SEM images of synthesized ZnO NPs using leaves extract of *Curcuma longa* at different magnifications (a) 40,000x (b) 50,000x (c) 20,000x

through $ZnSO_4$ showed good growth of root and shoot as the dose was increased although the germination percentage was decreased when the $ZnSO_4$ dose was increased while in case of nanoparticles the length of the shoot was first increased while decreased as the dose was increased, it showed greater growth and germination at each concentration then the bulk $ZnSO_4$, it showed maximum growth of shoot at 3 mg concentration while the pattern was almost constant in case of root length, there were not many changes in root length was seen while the root length was maximum at 3 mg concentration (Table-1). The visual representation of the growth of seedlings is shown in Fig. 5.

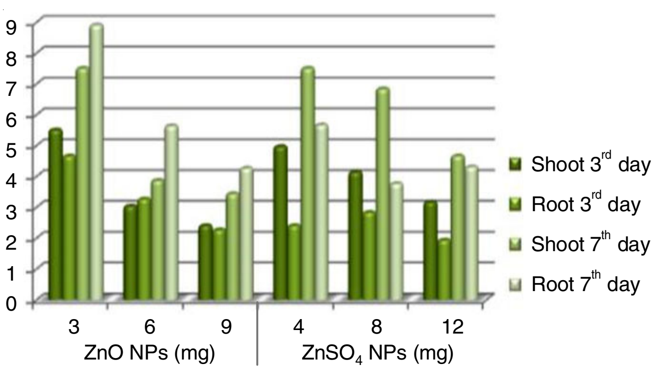

Fig. 4. Results of shoot and root length of mung bean seeds affected by different concentrations of $ZnSO_4$ and ZnO NPs

Total chlorophyll content: The total chlorophyll content of mung bean seeds treated with different concentrations of ZnO NPs, $ZnSO_4$ and control is shown in Fig. 6. The highest amount of total chlorophyll was found in mung bean treated with 3 mg concentration of ZnO NPs.

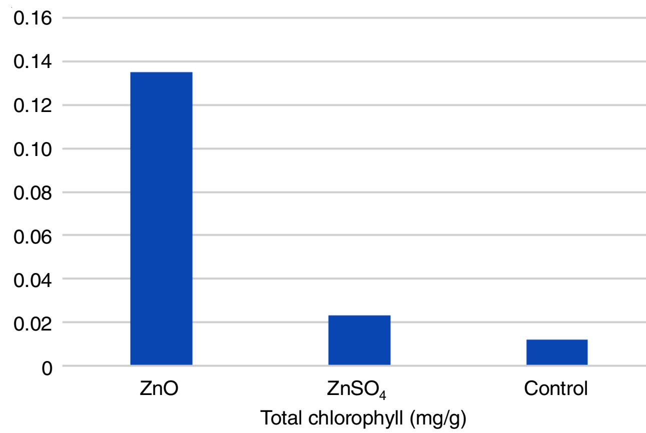

Fig. 6. Total chlorophyll content of mung bean seeds affected by ZnO NPs and $ZnSO_4$

Seedling vigour index (SVI): ZnO-NPs treated seeds were determined to be much better than the others. ZnO NPs at 3 mg had the greatest seedling vigour index of germinated seeds when

TABLE-1
EFFECT OF DIFFERENT CONCENTRATIONS IN GROWTH PARAMETERS OF DIFFERENT REPLICAS OF MUNG BEAN SEEDS AT DIFFERENT DAYS

Concentration	Shoot length (Mean ± SD) 3rd day	Root length (Mean ± SD) 3rd day	Shoot length (Mean ± SD) 7th day	Root length (Mean ± SD) 7th day	Germination (%)
$ZnSO_4$ (4 mg)	4.96 ± 0.55	2.40 ± 0.52	7.50 ± 0.65	5.66 ± 0.57	80
$ZnSO_4$ (8 mg)	4.13 ± 0.80	2.83 ± 0.60	6.83 ± 1.15	3.76 ± 0.60	60
$ZnSO_4$ (12 mg)	3.16 ± 0.30	1.93 ± 0.11	4.60 ± 1.52	4.30 ± 2.42	55
ZnO (3 mg)	5.50 ± 0.60	4.66 ± 0.76	7.50 ± 2.02	8.90 ± 1.00	98
ZnO (6 mg)	3.03 ± 0.40	3.26 ± 0.25	3.86 ± 0.73	5.63 ± 0.40	90
ZnO (9 mg)	2.40 ± 0.36	2.26 ± 0.35	3.43 ± 0.45	4.26 ± 0.41	70
Control	3.6	5.5	3	6	70

Fig. 5. Effect of different zinc treatments on *Vigna radiata* seedlings after 7 days

compared to other zinc treatments as shown in Fig. 7. Though varied levels of $ZnSO_4$ improved SVI, the magnitude of improvement was almost equal to that of their equivalent nano levels. It has been shown that low ZnO NPs concentration promotes seed root and shoot growth substantially (902.63). The plants growing using high concentration of ZnO NPs had a low seedling vigour index (311.415).

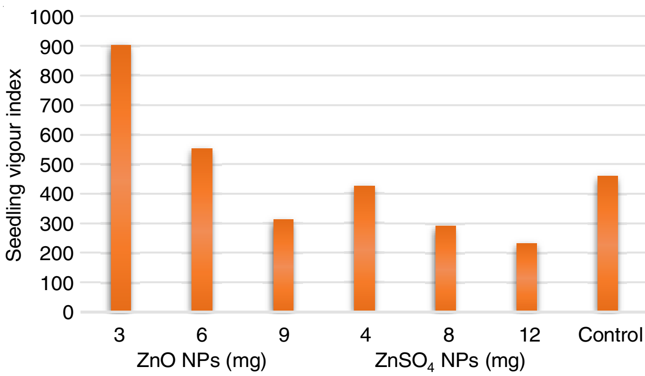

Fig. 7. Seedling vigour index (SVI) of mung bean seed affected by ZnO NPs, $ZnSO_4$ and control

Statistical analysis: The results were interpreted as mean standard deviation. The data collected after a 7-day period is shown in Table-2. Following the calculation of the mean, ANOVA was conducted to compare each experimental value with its corresponding control value, as reported by Hussain *et al*. [23]. The results indicated that the calculated f value exceeds the critical f value, as presented in Table-3, allowing us to reject the null hypothesis and conclude that there is a significant effect.

TABLE-2
DETAILS OF MUNG BEAN SEED GERMINATION % AND MEASUREMENT OF GROWTH PARAMETERS AT DIFFERENT DAYS

Conc.	Shoot length (cm) 3rd day	Root length (cm) 3rd day	Shoot length (cm) 7th day	Root length (cm) 7th day
$ZnSO_4$ (4 mg)	5.5	2.2	8.2	6.0
	5.0	2.0	7.4	5.0
	4.4	3.0	6.9	6.0
$ZnSO_4$ (8 mg)	5.0	2.2	7.9	4.0
	4.0	3.4	7.0	4.3
	3.4	2.9	5.6	3.0
$ZnSO_4$ (12 mg)	3.5	2.0	6.0	7.0
	3.1	1.8	5.0	2.3
	2.9	2.0	3.0	3.6
ZnO (3 mg)	5.8	4.0	9.8	7.9
	5.9	4.5	6.0	8.8
	4.8	5.5	6.7	10.0
ZnO (6 mg)	3.0	3.5	4.7	5.7
	2.6	3.3	3.3	5.2
	3.5	3.0	3.6	6.0
ZnO (9 mg)	2.7	2.6	3.4	5.8
	2.0	2.3	3.0	4.0
	2.5	1.9	3.9	3.0
Control	4.0	5.5	5.0	6.0

Conclusion

The influence of ZnO NPs synthesized using *Curcuma longa* leaves extract, on *Vigna radiata* L. seed germination and growth is studied. The *C. longa* leaves extract acts as both a stabilizing and reducing agent. For evaluation of the effect on mung bean seeds, nanoparticles were used along with the bulk $ZnSO_4$ at different concentrations. Zinc, in any form, has been found to have a favourable influence on seedling development when compared to the control. At a dosage of 3 mg/L, nanoparticles had the most favourable influence on germination of seed, root and shoot growth, total chlorophyll and seedling vigour index (SVI). However, when the concentration of zinc in the treatment increased, the growth rate reduced. At all concentrations, ZnO NPs outperform their bulk counterparts in terms of seedling growth. It was concluded that biosynthesized ZnO NPs had a favourable influence on the growth and germination of *Vigna radiata* and that using zinc in the form of nanoparticles may help in increasing the agricultural output. Thus, ZnO NPs can be utilized as a seed priming agent and potential fertilizer to enhance crop yields in the future. However, the response of plants to ZnO NPs was found to be dose-dependent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-3
STATISTICAL VALUES FOR THE GERMINATION OF MUNG BEAN SEEDS AFTER 7 DAYS PERIOD

Source of variation	SS	df	MS	F	P-value	F critical
Sample	104.22238100	6	17.37039683	21.84628437	0.73084×10^{-13}	2.265567389
Columns	86.01047619	3	28.67015873	36.05769327	0.12884×10^{-13}	2.769430949
Interaction	53.06619048	18	2.948121693	3.707773952	0.52531×10^{-5}	1.791157548
Within	44.52666667	56	0.795119048			
Total	287.8257143	83				

*The observed value is statistically significant

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