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Assessment of nanoparticle spray effects on lentil seed quality

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Abstract
Background: Nanotechnology is latest and new opportunity to develop our industry in the field of agriculture. In agriculture, it is suggested that foliar application of Zn and Cu nano particles offers available option for sustainable agriculture. The analysis was done to establish the impact of foliar nanotechnology on lentil crop seed quality under different stress conditions.
Methods: The experiment was performed in College of Forestry, Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry, Ranichauri, Tehri Garhwal, Uttarakhand, India in *rabi* 2021-22 under the temperate zone having 15 treatments and 4 replications. The complete randomized design was carried out to carry the investigation.
Result: The positive impact of 50% RDF along with nano Cu deploys on standard germination, speed of germination, mean daily germination, seed vigour indices, seedling length, seedling fresh and dry weight, cold test and water sensitivity test. In lentil seed, the statistically significant changes were noticed due to change in the conduction of stomata. The foliar application of 50% RDF + nano Cu confirms nanotechnology to be superior to traditional agriculture methods and is also environmentally friendly.

Keywords: Lentil, nano copper, nano zinc, cold test, water sensitivity test, standard germination

Introduction
Nanotechnology is new and emerging technique of novel industrial revolution with positive response on the agriculture production. As nanotechnology is not only science but art also due to the manipulation of substances and matter at nanoscale. It has immense potential to create agriculture at sustainable production and decreases the consumption of agrochemicals such as pesticide, fertilizers and insecticide etc. To feed the large population of our country, there is major demand to increase the production and productivity. The nanotool has enormous impact on the quantity and quality of agricultural production, and prevents an environmental health hazard (Sekhon, 2014; Liu and Lal, 2015) [15, 9]. Nanotechnology itself has unique properties as the nanoparticles boost the food quality, provide plant protection and improve seed quality. In case of nanoparticle application, there is an upgradation of agriculture production with high reactivity, better bioavailability, bioactivity and its effect on surface leads to better and alternative approach over traditional agriculture technique. The foremost benefit of nano technology is to improve the seed quality parameters in various crops. As the effect of nanoparticle depend and varies on plant to plant, species to species, crop to crop and seed to seed.
Lens culinaris Medic. is an winter annual leguminous crop with having second highest ratio of protein per calories of any legume after soybean (Mudryj *et al.*, 2014) [10]. It is a staple legume food that is traditionally grown in Indian subcontinent, West Asia, Eastern and Northern Africa. Lentil contributes positive impact of human and animal health which can be provided through nutrient to their body and simultaneously enhance and maintains the soil health.
As the nanoparticles have less diameters compared to pore of cell therefore it can be penetrated easily. Thus, foliar application is better to apply in the plants (Nair *et al.*, 2010) [11]. The legume crops are mainly deficient in micro nutrient like Zn, Cu, Fe, Ag, Si etc. But, deficiency of Cu and Zn has major effect on flowering and seed production. Researchers have revealed the

influence of Cu and Zn nanoparticles on crop like soybean, mung bean, pigeon pea, cow pea and broad bean at large scale but give less attention to lentil and interaction of Cu and Zn nanoparticle on lentil crop through foliar application. Here, the study is being conducted for the effect on Cu and Zn nano scale particles on seed germination, vigour and related parameters.

Materials and Methods

Application of Zn and Cu: The two foliar sprays of nano Zn @10000ppm and nano Cu @8000ppm was given at 30 and 90 DAS, as per the treatment *i.e.* 2ml/litre of water. Before application of the mentioned foliar spray, the recommended dose of fertilizers as per treatments was given through urea (46% N), DAP (16% N; 48% P) and MOP (60% K) at the time of sowing (November, 2020). For harvested crop (April, 2021) allowed to dry inside a netted cotton bag, plot wise separately, tagged, and were taken to the threshing floor for manual threshing by beating the plant material through wooden sticks. After this, grains were separated by winnowing from threshed materials and the seeds were kept in cloth bag, sun dried continuously till it reach optimum moisture level. For conducting seed quality analysis, 15 treatments have been conducted with four replications each. To investigate the seed quality of lentil crop the experiment was carried out in College of Forestry, Veer Chandra Singh Garhwali, Uttarakhand University of Horticulture and Forestry, Ranichauri, Tehri Garhwal, Uttarakhand, India in *rabi* 2021-22 and conducted in Complete Randomized Design (CRD). The details of treatments are given in Table 1.

The seed quality studies were observed through various parameters *viz.* standard germination (%), speed of germination, mean daily germination (%), seedling root and shoot length (cm), seedling length (cm), seedling fresh and dry weight with cotyledons (mg), seedling vigour index I and II, cold test and water sensitivity test.

The above mentioned parameters were calculated on 8^{th} day after sowing with different formulas.

$$Speed\ of\ germination = \frac{X1}{Y1} + \frac{X2 - X1}{Y2} + \cdots + \frac{Xn - Xn - 1}{Yn}$$

Where, X1 = Number of seeds germinated at first count
X2 = Number of seeds germinated at second count
Xn = Number of seeds germinated on n^{th} count
Y1 = Number of days from sowing to first count
Y2 = Number of days from sowing to second count
Yn = Number of days from sowing to n^{th} count

$$Mean\ Daily\ Germination(\%) = \frac{Final\ germination(\%)}{Total\ number\ of\ days\ in\ test}$$

$$Seed\ Vigour\ Index\ I = Standard\ germination\ (\%) \times Seedling\ length(cm)$$

$$Seed\ Vigour\ Index\ II = Standard\ germination\ (\%) \times\ Seedling\ dry\ weight\ (mg)$$

Cold test: The random samples of every treatment were taken with 50 seeds and kept in petri-dishes in germination chamber at 10˚C for 7 days. Then it was transferred to normal conditions and incubated for 8 days. Thereafter, the mentioned parameters were taken of 10 randomly selected seedlings.

Water sensitivity: Germination was tested in petri-dishes of 9 cm diameter, containing 70g dry sterilized sand to which 9 mL distilled water for low moisture condition and 18 mL distilled water for high moisture condition is added (Mathews and Collins, 1975). The dishes were incubated for 8 days at 20±1˚C after sowing having 20 seeds per dish at a depth of 0.5 cm. Thereafter, the mentioned parameters were taken of 10 randomly selected seedlings.

Statistical analysis: The analysis was done by OP Stat for CRD by O.P. Shearon Programmer, Computer section, CCS HAU, Hisar.

Results

All the parameters *viz.*, standard germination, mean daily germination, speed of germination, seedling root and shoot length, seedling length, seedling fresh and dry weight with cotyledons exerted significant variations among all the treatments in lentil. The treatment 50% RDF + Nano Cu recorded maximum of all the mentioned parameters which was superior to all other remaining treatments. Speed of germination continuously increases with the advancement in seedling growth and reached maximum at 5^{th} day being constant thereafter. The treatment 50% RDF + Nano Cu was found on par with 50% RDF + Nano Zn + Nano Cu for all the parameters (Fig. 1 to 3). This variation also exerted significant effect on seedling vigour index I and II. The treatment 50% RDF + Nano Cu recorded significantly maximum seed vigour index I and II that was statistically superior to all other treatments (Fig. 4).

Cold test: The result of the various germination parameters under cold stress condition are illustrated in Table 2. The treatment 50% RDF + Nano Cu recorded maximum standard germination, which outperformed all other treatments significantly being at par with 50% RDF + Nano Zn + Nano Cu. The treatments 50% RDF + Nano Cu and 50% RDF + Nano Zn + Nano Cu recorded equivalent and significantly maximum mean daily germination under cold test being at par with 75% RDF + Nano Zn compared to all other treatments. All the treatments exerted significant variation in the seedling shoot, root and seedling length being maximum in 50% RDF + Nano Cu and statistically at par with 50% RDF + Nano Zn + Nano Cu. The treatment 50% RDF + Nano Cu recorded maximum seedling fresh and dry weight of cotyledons under cold test was significantly superior to all other treatments and ultimately recording maximum seed vigour index I and II under cold test.

Water Sensitivity: All the treatments exerted significant variation in the seed quality of lentil under water sensitivity test. The treatment 50% RDF + Nano Cu recorded maximum performance in water sensitivity test both in high and low moisture condition, which demonstrated superior statistical significance compared to all other treatments in terms of seedling fresh and dry weight and seedling length (Fig. 5 and 6).

Discussion

Germination and seedling establishment are the one of the most critical part in the life of plant. Along with seed germination, water absorption, enzymatic activity, growth of embryo, break of seed coat, seedling establishment, cold stress and water sensitivity test of the resulted experiment are discussed. Seed quality parameters such as speed of germination, standard germination percentage, mean daily germination, seedling shoot length, seedling root length, seedling length, seedling fresh weight and seedling dry weight with cotyledons, seed vigour index I and II under standard germination were recorded highest in 50% RDF + Nano Cu. This might be due to the earliness in the germination caused by the enhanced metabolites and their activity, which helps in resumption of embryonic growth during germination. The physiological performance was enhanced due to the nanoparticles through the abating of free radicals. The smaller size of the particles leads to better penetration within the cell resulted enhancement in seed germination and vigour (Sharma *et al.*, 2018) [16]. The similar response was founded by Singh *et al.* (2017) [17] on mungbean, Prasad *et al.* (2012) [12] on peanut, Korishettar *et al.* (2016) [8] in pigeon pea and Bhanupriya *et al.* (2020) [1]. The nanoparticles copper and zinc are engaged in triggering amylase and protease enzymes associated with the mobilization of nutrients for seed germination, thereby enhancing germination rates (Choudhary *et al.*, 2017) [2] and similar view was revealed by Sandeep *et al.* (2019) [13] on treating soybean seed with nanoparticles.

Under the stress in cold test, 50% RDF + Nano Cu performed better again for standard germination, seedling shoot length, seedling root length, seedling length, seedling fresh weight and seedling dry weight with cotyledons might be due to the foliar spray of nano Cu that increased the respiration rate and metabolic rate there by reducing the stored food material of the seed and loss of integrity of the membrane (Daniel *et al.*, 2018) [3], which promote the seedling growth. The significant differences were recorded due to the stomatal conductance and transpiration efficiency and close conformity with Kolencik *et al.*, 2019; Kolencik *et al.*, 2020. The stress experienced was caused by changed environmental conditions, and NP intervention led to heightened plant resilience. The higher control cold test and water sensitivity indicate that Cu and Zn nano particles application eliminate the effect of environmental stress on the lentil plant, including its response to water-deficiency and higher air-temperatures (Franks and Brodribb, 2005; Kirkham, 2014) [4, 5]. Under the water sensitivity it is the main reason behind to increase the seedling length, seedling dry weight and fresh weight in lentil.

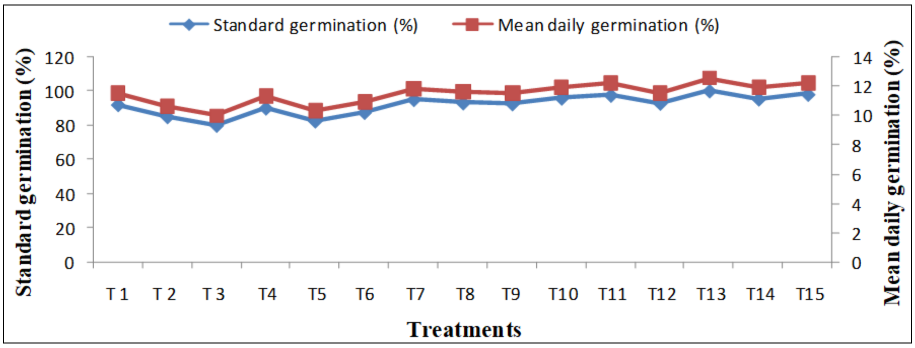

Fig 1: Effect of foliar application of nano scale zinc and copper on standard germination and mean daily germination of lentil

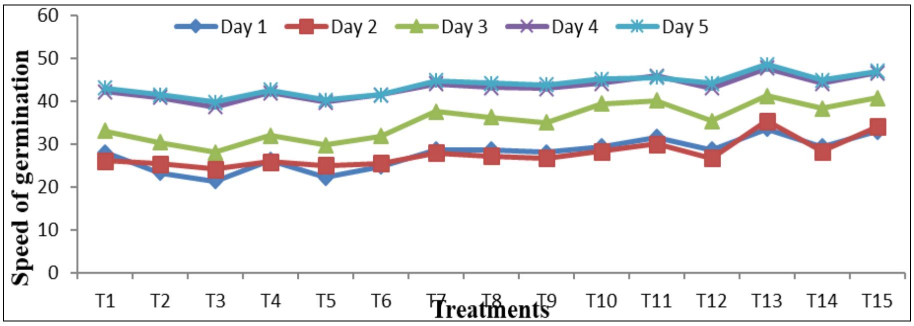

Fig 2: Effect of foliar application of nano scale zinc and copper on speed of germination of lentil

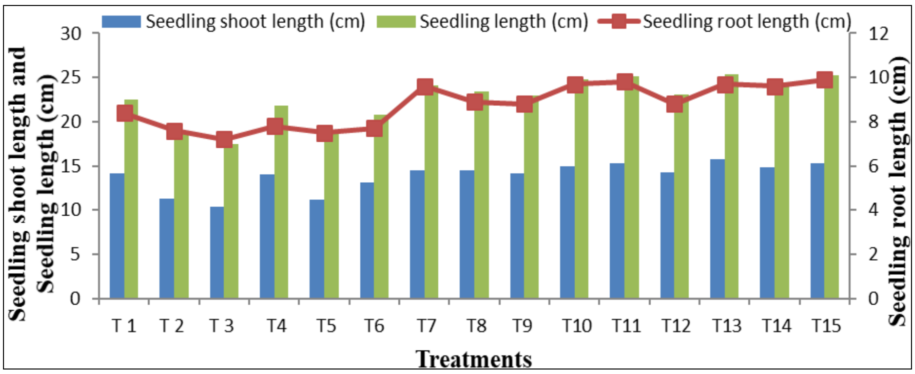

Fig 3: Effect of foliar application of nano scale zinc and copper on seedling shoot length, seedling root length and seedling length of lentil under standard germination test

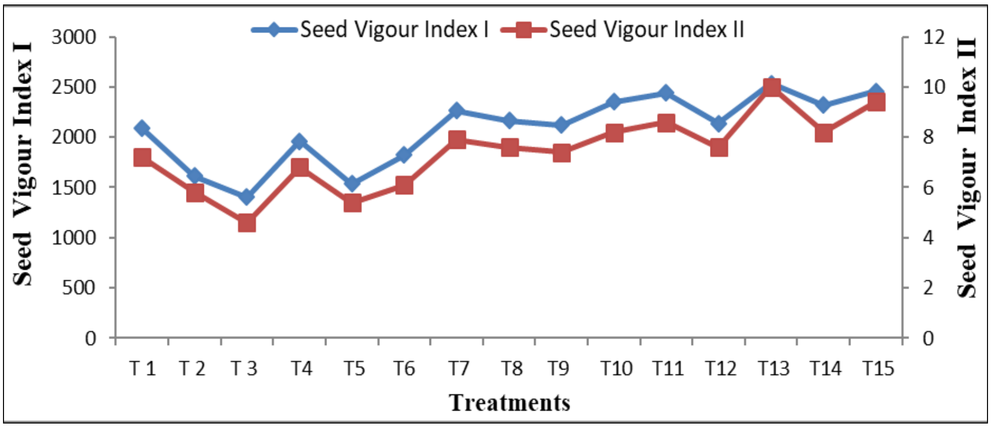

Fig 4: Effect of foliar application of nano scale zinc and copper on seed vigour index I and II of lentil under standard germination test

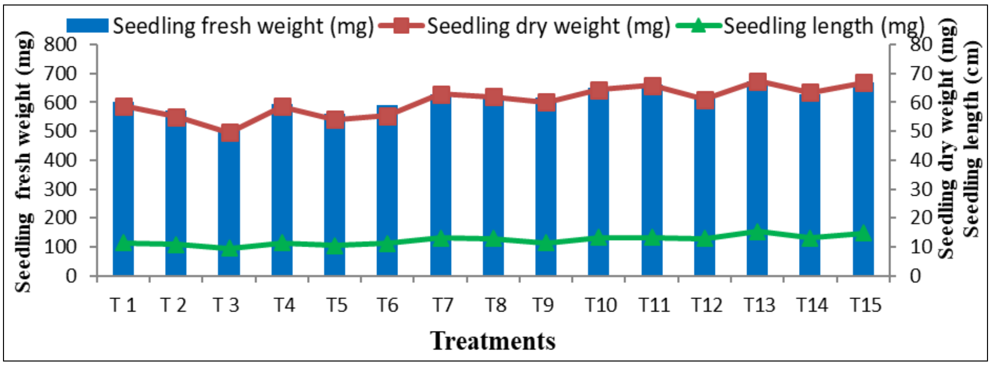

Fig 5: Effect of foliar application of nano scale zinc and copper on seed quality of lentil under water sensitivity test in high moisture condition

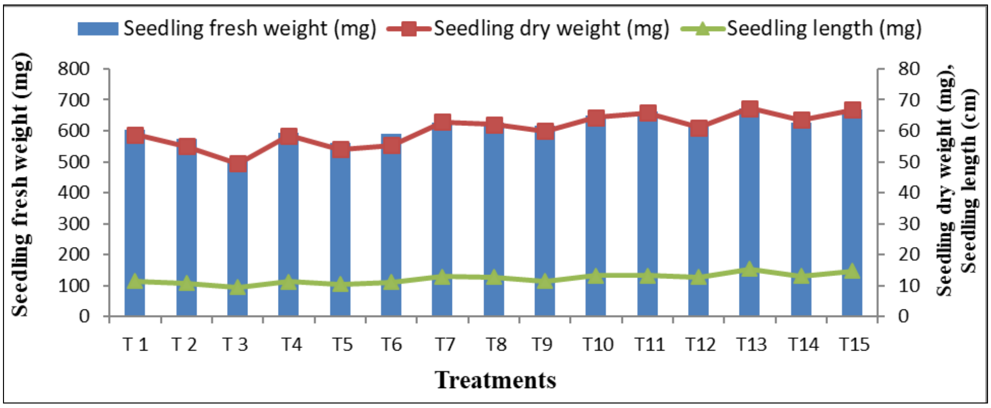

Fig. 6: Effect of foliar application of nano scale zinc and copper on seed quality of lentil under water sensitivity test in low moisture condition

Table 1: Treatment details

S. No.	Treatments	Symbol used
1.	100% RDF (20:40:20 kg N:P:K/ha)	T_1
2.	75% RDF (15:30:15 kg N:P:K/ha)	T_2
3.	50% RDF(10:20:10 kg N:P:K/ha)	T_3
4.	Nano Cu(2ml l^{-1})	T_4
5.	Nano Zn (2ml l^{-1})	T_5
6.	Nano Zn + Nano Cu(2ml l^{-1} each)	T_6
7.	100% RDF + Nano Cu (2ml $l^{-1)}$	T_7
8.	100% RDF + Nano Zn (2ml $l^{-1)}$	T_8
9.	100% RDF + Nano Zn + Nano Cu (2ml l^{-1} each)	T_9
10.	75% RDF + Nano Cu (2ml l^{-1})	T_{10}
11.	75% RDF + Nano Zn (2ml l^{-1} each)	T_{11}
12.	75% RDF + Nano Zn + Nano Cu (2ml l^{-1} each)	T_{12}
13.	50% RDF + Nano Cu (2ml l^{-1})	T_{13}
14.	50% RDF + Nano Zn (2ml l^{-1})	T_{14}
15.	50% RDF + Nano Zn + Nano Cu (2ml l^{-1} each)	T_{15}

Table 2: Effect of foliar application of nano scale zinc and copper on seed quality of lentil under cold test

S. No.	Treatments	Cold test								
		Standard germination (%)	Mean daily germination (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling length (cm)	Seedling fresh weight with cotyledons (mg)	Seedling dry weight with cotyledons (mg)	Seed Vigour Index I	Seed Vigour Index II
T_1	100% RDF	91.3	6.5	22.7	11.8	34.4	960.0	62.5	3141.0	5.7
T_2	75% RDF	89.5	6.4	22.2	11.2	33.4	922.5	55.0	2991.0	4.9
T_3	50% RDF	87.5	6.3	21.4	10.8	32.1	905.0	52.5	2825.0	4.6
T_4	Nano Cu	90.8	6.5	22.6	11.5	34.1	945.0	60.0	3093.0	5.4
T_5	Nano Zn	88.8	6.3	22.0	11.2	33.2	915.0	55.0	2938.0	4.9
T_6	Nano Zn + Nano Cu	90.0	6.4	22.2	11.3	33.5	925.0	57.5	3020.0	5.2
T_7	100% RDF + Nano Cu	94.5	6.8	23.6	12.5	36.1	1035.0	75.0	3408.0	6.9
T_8	100% RDF + Nano Zn	93.8	6.7	23.4	12.4	35.8	1030.0	70.0	3354.0	6.6
T_9	100% RDF + Nano Zn + Nano Cu	92.5	6.6	22.7	12.0	34.8	967.5	65.0	3214.0	6.0
T_{10}	75% RDF + Nano Cu	97.0	6.9	24.2	13.7	37.9	1090.0	75.0	3648.0	7.3
T_{11}	75% RDF + Nano Zn	97.5	7.0	24.4	13.7	38.1	1147.5	77.5	3718.0	7.5
T_{12}	75% RDF + Nano Zn + Nano Cu	93.3	6.7	23.1	12.0	35.1	992.5	67.5	3271.0	6.4
T_{13}	50% RDF + Nano Cu	100.0	7.1	25.2	14.0	39.2	1275.0	87.5	3923.0	8.8
T_{14}	50% RDF + Nano Zn	95.0	6.8	24.1	12.7	36.8	1037.5	72.5	3491.0	7.1
T_{15}	50% RDF + Nano Zn + Nano Cu	98.8	7.1	24.8	13.8	38.7	1152.5	80.0	3815.0	7.9
	S.Em±	5.6	0.4	1.4	0.6	1.9	29.4	2.1	57.4	0.1
	CD (P = 0.01)	1.9	0.1	0.5	0.2	0.7	10.3	0.7	81.2	0.1

Conclusion
The result of this study illustrate that Cu and Zn nano scale particles can directly and indirectly effect the germination, seed vigour indices, can provide better strength for plant to survive under stressful conditions. It can be concluded that 50% RDF + nano Cu increases the seed quality parameters. It can also used to facilitate the plant growth, maintain the seed quality and ultimately raise the performance of crop.

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