International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 1786-1791

Received: 01-07-2019
Accepted: 03-08-2019

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Studies on efficacy of nanoparticles in improving seed physiological parameters in groundnut (*Arachis hypogaea* L.)

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Abstract
An investigation was initiated to examine the efficacy of nanoparticles in improving seed physiological parameters and seed quality in groundnut during 2018-19. Groundnut seeds were separately treated with different concentrations of nanoparticle. Seed treatment with nano ZnO @ 500 ppm significantly promoted seed quality attributes like germination (76.75%), root length (13.78cm), shoot length (7.43cm), SVI I (1627.20), and SVI II (279.22), alpha amylase activity (23.15mm) also differed significantly in the treatments compared to that of the control. These results indicate that the nano ZnO @ 500 ppm have significant effects on the growth and development for enhancement of agricultural crops particularly in groundnut and also improves the zinc content in seeds which is an utmost important feature in terms of human health perspective.

Keywords: Groundnut, nanoparticles, seed quality and seed yield

Introduction

The groundnut also known as the peanut, taxonomically named as *Arachis hypogaea* L. is a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. It is classified as both a grain legume and oil seed crops because of its high oil content India stands first both in area and production of groundnut but, productivity is low. This is attributed by factors such as cultivation of the crop on marginal and sub-marginal lands (mainly under rain fed condition), uncertain monsoon leading to frequent drought, poor adaption of improved agronomic practices, use of low yielding and late maturing varieties, wide spread losses due to pest and diseases, inadequate availability of quality seeds and many socio-economic factors.

As the current technologies available to prolong the vigour and viability of groundnut seed on a large scale are not satisfactory alleviating the practical problem, an alternative simple and practicable seed treatment methodology/technique to control seed deterioration of groundnut seeds is the need of the hour. During the past decade, lot of work has been done in biological system to address a wide range of field problems utilizing nanomaterials and nano-devices.

A number of researchers have reported the essentiality and role of zinc for plant growth and yield (Chapman, 1966; Viets, 1966) [4, 15]. In India, Zn is now considered the fourth most important yield limiting nutrient after nitrogen (N), phosphorus (P), and potassium (K). However, in India around 50 per cent of the groundnut growing soils are reported as Zn deficiency, which is causing considerable yield loss (Singh, 1999) [13]. Considerable increases in grain yield of groundnut by Zn application was also demonstrated in India (Tandon, 1998) [14]. Zinc and iron is required for chlorophyll production, pollen function, fertilization, germination and biomass production.

Unfortunately, due to lack of improved post harvest preservation technique, a large proportion of seed was gets lost in storage. However, it can be controlled to certain extent by adopting new technologies. Nanotechnology is an emerging technology and promises substantial help to agriculture which can lead a new revolution. Nano SiO_2 and nano TiO_2 increases nitro reductase, also increases the seed germination and growth in groundnut crop. Nanoparticles with small size and large surface area are expected to be the ideal candidates for use as a fertilizer in plants. Farmers are using both sulphate and chelated (with ethylene diammine tetraacetic acid, EDTA) for soil and foliar applications; however, the efficacy is low.

There are very few research conducted on concentration of nanoparticles to improve the yield and quality of groundnut seeds. The present study considered to know the inhibitory effects of various concentrations of nanoparticles on growth, development yield and quality of groundnut (*Arachis hypogaea* L).

Material and methods

In this experiment, seeds were treated using seed drum coating machine with for five different nanoparticles along with 8 ml polymer and dried under shade for (24 h) for the safe moisture content. The details of the treatments are furnished below.

Treatment Details

T_1: 8 ml polymer (control)
T_2: 8 ml polymer + ZnO @ 500 ppm
T_3 :8 ml polymer + ZnO@ 1000 ppm
T_4: 8 ml polymer + ZnO@ 1500ppm
T_5: 8 ml polymer + FeO@ 200 ppm
T_6: 8 ml polymer + FeO@ 400 ppm
T_7: 8 ml polymer + FeO@ 600 ppm
T_8: 8 ml polymer + SiO_2 @ 25 ppm
T_9: 8 ml polymer + SiO_2@ 50 ppm
T_{10}: 8 ml polymer + SiO_2 @ 75 ppm
T_{11}: 8 ml polymer + CNT@ 25 ppm
T_{12}: 8 ml polymer + CNT@ 50ppm
T_{13}: 8 ml polymer + CNT@ 75 ppm
T_{14}: 8 ml polymer + TiO_2@ 50 ppm
T_{15}: 8 ml polymer + TiO_2@ 100ppm
T_{16}: 8 ml polymer + TiO_2@ 150ppm

Seed germination (%)

The germination test was carried out in eight replicates of fifty seeds in each treatment, temperature in seed germinator was maintained at 25± 1 ºC and 90± 2 per cent RH using the 'between paper' method as per ISTA, (2013) rules. The number of normal seedlings was counted on 10th day of germination test and the average of four replications was worked out and expressed as percentage based on normal seedlings.

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Root length (cm)

Observations from the germination test, ten normal seedlings were randomly selected from each replication of the standard germination test on the 10th day and length of the root was measured from collar region down to the tip of the longest root of each seedling and the average was expressed in centimeter.

Shoot length (cm)

Observations from the germination test, ten normal seedlings were selected at random from each replication on tenth day and the length of shoot was measured from the collar region to the tip of coleoptile and the average shoot length was expressed in centimeter.

Seedling dry weight (mg)

Seedling dry weight was estimated in four replications following the standard method (Gupta, 1993). Ten normal seedlings were picked randomly from the germinated seeds from each replication. The seedlings were wrapped in butter paper and kept in oven at 70±2 for 24 h. The dry weight was recorded after cooling in a desiccator containing silica gel. Mean seedling dry weight was expressed in milligrams.

Seedling vigour index I

The germinated seedlings were evaluated on 5th and 10th day as first and final count, respectively. The percentage of germination was expressed based on the normal seedlings present in the test. Ten normal and healthy seedlings from each replication were selected randomly on 10th day and seedling length (shoot and root) was measured in centimeter. Then the Seedling Vigour Index I was determined by multiplying standard germination (%) and mean seedling length (cm) and expressed in number (Abdul-Baki and Anderson, 1973) [1].

Seedling vigour index I = Germination (%) × Mean seedling length (cm)

Seedling Vigour Index II

The five seedlings selected randomly for germination test for calculating the Seedling Vigour Index-II were oven dried after removing the cotyledon (remnant seed) and the mean seedling dry weight of these seedlings was used for calculating the Seedling Vigour Index II by using the formula given by Abdul Baki and Anderson (1973) [1] as indicated below:

Seedling vigour index II = Germination (%) x Mean seedling dry weight (mg)

Alpha amylase activity (mm)

The α-amylase activity was analyzed as per the method suggested by Simpson and Naylar (1962) [12].

Results and Discussion

The effect of nanoparticles seed treatment with different concentrations varied significantly on germination (%). Among the nanoparticles observed the ZnO @ 500 ppm had recorded the highest germination percentage (76.75%). It is on par with the FeO @ 200 ppm (75.75%), SiO_2 @ 25 ppm (74.25%), CNT @ 75 ppm (73.00%), TiO_2 @ 50 ppm (72.25%). It was significantly superior over 8 ml polymer control which is having lowest germination percentage (68.00%). Above results are in conformity with Prasad *et al.* (2012) [11] reported improvement in germination, root growth, shoot growth, dry weight and pod yield in groundnut due to seed treatment with ZnO nanoparticles @ 500 ppm. In *Cicer* arietinum, Avinash *et al.* (2010) [3] also found that ZnO NPs increased the level of IAA in the roots (sprouts) and thereby an increase in the growth rate of plants. According to Kobayashi and Mizutani (1970) [8] the beneficial effect of the ZnO NPs in improving the germination could be ascribed to higher precursor activity of nanoscale zinc in auxin production. Besides this zinc is also one of the essential nutrients required for plant growth, being an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Zinc oxide NPs are reported to exhibit positive effect on the reactivity of phytohormones especially Indole acetic acid (IAA) facilitates in phyto stimulatory actions which dramatically regulates the plant growth. The improvement of Zn nutritional status also reduces the uptake of harmful heavy metals that hinders its toxicity in plants (Adiloglu, 2002) [2].

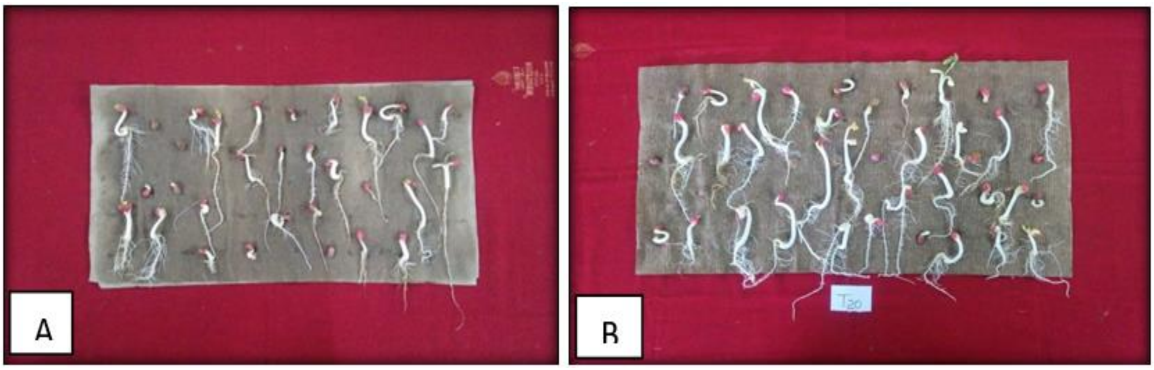

Fig 1: Effect of nanoparticle treatments on seeds treated with a) 8 ml polymer b) ZnO @ 500 ppm on germination (%).

There is a huge varation were noticed among the treatments studied with respective to shoot length. Among the treatments ZnO@500ppm which has given the greater effect in comparison with all treatments (7.43cm). Where as it is showing on par with the FeO @ 200ppm (7.20cm), SiO_2@ 25ppm (6.60cm), CNT @ 75ppm (6.40cm) TiO_2 50ppm (6.40cm) (lowest shoot length were recorded in the polymer control (4.35cm). The probable reason for enhanced physiological performance due to nanoparticles treatment could be attributed to the quenching of free radicals by the nanoparticles. Smaller size of the nanoparticles would have easily entered through cracks present on the outer seed surface, reacted with free radicals resulting in enhanced seed vigour. (Sen gupta *et al.*, 2005).

The effect of nanoparticles seed treatment with different concentrations varied significantly for root length. The results revealed there is a variation among the nanoparticles treatments studied. Among them ZnO@ 500 ppm treated seeds shown maximum root length (13.8 cm) Were it is showing on par with FeO 200 ppm (13.6 cm) and SiO_2@ 25ppm (12.9 cm), CNT@ 75ppm (12.6cm), TiO_2 @ 50ppm (12.5cm). The lowest root length was recorded in polymer control (9.2 cm). It might be due to nanoparticles would induce oxidation reduction reactions via the superoxide ion radical during germination, resulting the quenching of free radicals in the germinating seeds. In turn, oxygen produced in such process could also be used for respiration, which would further promote germination (Zheng *et al.*, 2006).

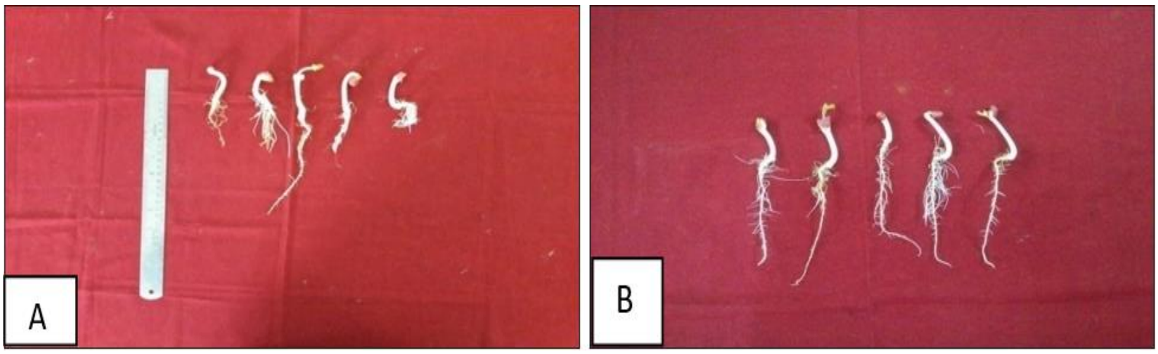

Fig 2: Effect of nanoparticle treatments on root length and shoot length (cm) for 8 ml polymer control b) ZnO @ 500 ppm.

The effect of nanoparticles seed treatment with different concentrations varied significantly for seedling dry weight. The results revealed that there is a wide variation among the nanoparticles treatments studied. Among the nano particles, ZnO @500 ppm NPs treated seeds had maximum seedling dry weight (3.64 mg). It is on par with the FeO @ 200 ppm (3.57 mg), SiO_2 @ 25 ppm (3.40 mg), CNT @7 5 ppm (3.34mg), TiO_2 @ 50 ppm (3.31 mg). Lowest seedling dry weight was recorded in polymer control (2.57 mg). The results are in accordance with that of the Zheng *et al.* (2005) best results were found at 60 ppm silver nanoparticles, the fresh weight and dry weight per plant were higher than those of the control by 30 % and 27 %, respectively for common bean. For corn fresh weight and dry weight per plant were higher than those of the control by 35 % and 33 %, respectively.

Among the nano particles, ZnO@500 ppm which has given the greater impact on seedling vigour index I (1627.20). It is found on par with FeO NPs (1576.98) and SiO_2@ 25 ppm (1445.10), CNT @ 75 ppm (1503.80), and TiO_2 @ 50 ppm (1461.60), and the lowest seedling vigour index I was recorded in polymer control (918.18). Enhanced physiological performance due to nanoparticles treatment could be attributed to the quenching of free radicals by the nano particles. Smaller size of the nanoparticles would have easily entered through the cracks present on the outer seed surface, reacted with free radicals resulting in reducing damage to the biological system and might have caused enhanced seed vigour and viability.

The effects of nanoparticles seed treatments, at different concentrations were found to be significant for alpha amalyase activity. Among the nano particles, ZnO@ 500 ppm which has given the greater effect on alpha amalyase. ZnO @ 500 ppm (23.15 mm), followed by FeO @ 200 ppm (22.85mm), SiO_2 @ 25 ppm (22.61mm), CNT @ 75 ppm (21.62 mm), TiO_2 @ 50 ppm (21.6 mm). Lowest alpha amylase activity was recorded in polymer control (17.17 mm). It is evident from the results that among the Zn and Fe, the Zn is the most critical micronutrient which influences the amylase activity. The α-amylase activity was significantly increased by Zn NPs at 500 and 1000 ppm over other treatments. This could be due to the increased availability of Zn at nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the- amylase enzyme.

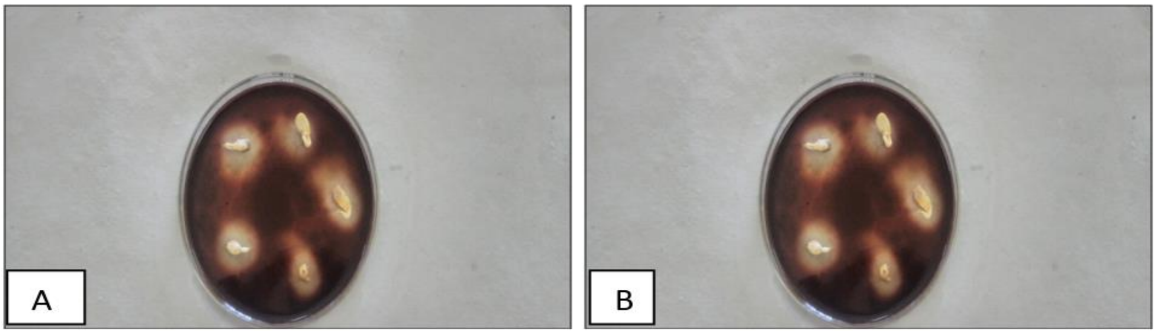

Fig 3: Effect of nanoparticle treatments on alpha amylase activity for 8 ml polymer control b) ZnO @ 500 ppm

The effects of nanoparticles seed treatments in pot culture, at different concentrations were found to be significant for plant height at 90 DAS. Among the nanoparticles, ZnO @ 500 ppm which has given the greater impact on plant height (45.8 cm). It is on par with FeO@ 200 ppm (43.3 cm) and lowest plant height (32.0 cm) was lowest in polymer control. The results are in agreement with that the mechanism of foliar uptake pathway for aqueous solutes and water suspended nanoparticles was well discussed by Eichert *et al.* (2008) [6] in the context of *Allium porrum* and *Vicia faba* (L.). The results suggested that the, stomatal pathway differ fundamentally from the cuticular foliar uptake pathway. Low penetration rates in thick leaves, rapid drying of spray solution, limited translocation within the plant, and leaf damage are the problems of concern (Marschner, 1995) [9] and most foliar applied micronutrients are not efficiently transported towards the roots. Concentrated liquid suspensions of Zn oxide nano particle were used for foliar application but their performance is strongly determined by the size range specification of the ZnO particles present in the formulation (Moran, 2004) [10]. Leaf water repellency of adaxial or abaxial surface is a main limiting factor, which can affect the Zn uptake through spray application processes (Watanabe and Yamaguchi, 1991; Holder, 2007) [16, 7].

Number of pods per plant differed significantly due to application of different nanoparticles at different concentration. Significantly highest numbers of pods per plant (16)were recorded in ZnO @ 500 ppm followed by ppm FeO @ 200ppm (15), SiO_2 @ 25 ppm (14), CNT @75 ppm (14) and TiO_2 @ 25ppm (13). Lowest number of pods was recorded in control (11). The results are in accordance with that the permeability of the cuticle to water and to lipophilic organic molecules increases with mobility (distribution co-efficient) and solubility (partition co efficient) of these compounds within the transport-limiting barrier of the cuticles. Ions being highly water soluble might have some hindrance in penetrating the lipophilic cuticle. This may be acting as a limiting factor in the case of chelated ZnO. But our custom-made nano scale ZnO which is having less hydrophilic and being more dispersible in lyophilic substances compared to the ions, can penetrate through the leaf surface (Da Silva *et al*., 2006) [5] compared to bulk ZnO and also the mobility of the nanoparticles is known to be very high which ensures the phloem transport and also the nutrient movement to all parts of the plant.

Significantly highest number of seed yield per plant was recorded in ZnO @ 500 ppm (26.00 g). It was found on par with FeO @ 200 ppm (25.74). However, lowest number of seed yield per plant was recorded in polymer control (23.11g). Most of the physiological parameters like plant height, number of leaves, number of tillers, chlorophyll content, higher grain yield and higher biomass recorded. The Zn content in the edible parts of crop plants like leaf, root, stem and seeds, in the study with nano Zn treatment either only seed / foliar treatment or seed priming with foliar application and higher showed a significant positive response. Nevertheless, all the Zn treated plants showed significantly affirmative response compared to the untreated control plants which explains the physiological importance of Zn in plant metabolism.

Table 1: Effect of different concentrations of nanoparticles on the normal seedlings (%) root length (cm) and shoot length (cm) in groundnut var. Kadiri -9

Treatments	Normal seedlings (%)	Root length (cm)	Shoot length (cm)
T_1: 8 ml polymer control	64.00	9.18	4.35
T_2: 8 ml polymer + ZnO 500 ppm	76.75	13.78	7.43
T_3: 8 ml polymer + ZnO 1000 ppm	68.75	10.75	5.28
T_4: 8 ml polymer + ZnO 1500 ppm	70.25	12.03	6.10
T_5: 8 ml polymer + FeO 200 ppm	75.75	13.63	7.20
T_6: 8 ml polymer + FeO 400ppm	68.00	12.13	5.93
T_7 : 8 ml polymer + FeO 600 ppm	72.00	12.10	5.98
T_8 : 8 ml polymer + SiO_2 25 ppm	74.25	12.85	6.60
T_9 : 8 ml polymer + SiO_2 50 ppm	66.50	11.68	6.53
T_{10} : 8 ml polymer + SiO_2 75 ppm	65.50	10.35	5.03
T_{11} : 8 ml polymer + CNT 25 ppm	69.00	12.05	5.33
T_{12} : 8 ml polymer + CNT 50ppm	70.00	11.98	4.20
T_{13} : 8 ml polymer + CNT 75 ppm	73.00	12.60	6.45
T_{14} : 8 ml polymer + TiO_2 50 ppm	72.25	12.45	6.40
T_{15} : 8 ml polymer + TiO_2 100 ppm	69.75	12.25	5.90
T_{16} : 8 ml polymer + $TiO_2$150 ppm	70.00	11.48	5.25
Mean	70.36	11.95	5.87
SEm ±	1.16	0.22	0.30
CD@1%	4.78	0.84	1.12

Table 2: Effect of different concentrations of nanoparticles on the seedling dry weight (g) seedling vigour index I and seedling vigour index II in groundnut var. Kadiri -9

Treatments	Seedling dry weight(g)	Seedling vigour index I	Seedling vigour index II
T_1: 8 ml polymer control	2.57	918.18	164.99
T_2: 8 ml polymer + ZnO 500 ppm	3.64	1627.20	279.22
T_3: 8 ml polymer + ZnO 1000 ppm	3.16	1101.48	216.91
T_4: 8 ml polymer + ZnO 1500 ppm	3.03	1272.88	212.50
T_5: 8 ml polymer + FeO 200 ppm	3.57	1576.98	270.82
T_6: 8 ml polymer + FeO 400ppm	2.81	1228.00	191.31
T_7 : 8 ml polymer + FeO 600 ppm	2.97	1302.50	213.63
T_8 : 8 ml polymer + SiO_2 25 ppm	3.40	1445.10	251.91
T_9 : 8 ml polymer + SiO_2 50 ppm	2.94	1212.55	195.83
T_{10} : 8 ml polymer + SiO_2 75 ppm	2.84	1003.30	186.08
T_{11} : 8 ml polymer + CNT 25 ppm	2.87	1282.25	198.47
T_{12} : 8 ml polymer + CNT 50ppm	2.88	1133.68	201.75
T_{13} : 8 ml polymer + CNT 75 ppm	3.34	1503.80	243.89
T_{14} : 8 ml polymer + TiO_2 50 ppm	3.31	1461.60	238.79
T_{15} : 8 ml polymer + TiO_2 100 ppm	3.29	1266.85	229.26
T_{16} : 8 ml polymer + $TiO_2$150 ppm	3.27	1170.85	229.48
Mean	3.12	1281.70	220.30
SEm ±	0.13	86.62	11.80
CD@1%	0.54	328.58	44.75

Table 3: Effect of different concentrations of nanoparticles on number of pods seed yield per plant (g) and test weight (g) in groundnut var. Kadiri -9

S. No	Treatments	No. of pods per plant	Seed yield per plant (g)	Test weight (g)
1	T_1: Control	11	23.11	25.22
2	T_2: ZnO 500 ppm	16	26.00	30.63
3	T_3 : FeO 200 ppm	15	25.74	29.63
4	T_4: SiO_2 25 ppm	14	24.71	28.46
5	T_5: CNT 75 ppm	14	24.40	27.41
6	T_6: TiO_2 50 ppm	13	24.05	27.07
	Mean	14	24.67	28.07
	SEm ±	0.67	0.28	0.46
	CD @5%	1.98	0.84	1.36

Conclusion

It may be concluded from the research (or) from the above study that the seed treated with ZnO @ 500 ppm has increasing in the seed physiological parameters like plant height (45.8cm), number of primary branches (7), number of secondary branches (6), and seed yield attributing characters are also influenced with this nanoparticles namely seed yield per plant, pod yield per plant.

Thus the study suggested that both physiological and biochemical parameters did not vary considerably between pot culture experiment and the laboratory conditions. Therefore, seed treatment with ZnO @ 500 ppm can be recommended for achieving higher seed yield and quality in ground nut.

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