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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 2246-2250

www.thepharmajournal.com
Received: 27-07-2023
Accepted: 30-08-2023

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Evaluating the impact of iron oxide nanoparticles on nutritional parameters and yield attributes of groundnut grown in calcareous soils

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Abstract
Nanotechnology is an advanced science that has the ability to mitigate a myriad of problems. In agricultural systems, it has been shown to be of use as nanofertilisers, nanopesticides and nano-biosensors. This experiment was designed to understand the impact of different concentrations of iron oxide nanoparticles on the nutritional and yield attributes of groundnut grown in calcareous soils. The different treatments used were: T_1: Foliar application with $FeSO_4$, T_2: Control, T_3: Foliar application with iron nanoparticles @ 25 ppm, T_4: Foliar application with iron nanoparticles @ 50 ppm, T_5: Foliar application with iron nanoparticles @ 75 ppm. Significant results were obtained with the treatment of 50 ppm nanoparticles suggesting the efficacy of these nanoparticles as a corrective factor to mitigate iron deficiency in the growth of groundnut in calcareous soils.

Keywords: Nanotechnology, iron deficiency, groundnut, nutrition

1. Introduction

Groundnut is a leguminous crop that contains a magnitude of dietary sources of protein, essential fatty acids, vitamins such as: Vitamin B1, Vitamin B2, Niacin, Vitamin B6, Folate, calories and extensive range of minerals like: potassium (K), sodium (Na), calcium (Ca), iron (Fe), magnesium (Mg) and phosphorus (P) and it has shown a direct correlation with numerous health benefits (Ojiewo *et al*., 2020) [15]. Apart from its broad use of groundnut seeds as edible oil, the kernels can also be consumed in raw roasted form, boiled, as peanut butter, coated and fried kernel, used in cookies, curries, and even as bar in form of *chikki* (Varela and Fiszman, 2011) [21]. The major groundnut producing countries are: China, India, Nigeria, Sudan, USA, Myanmar. In India, groundnut is extensively grown in states like: Gujarat, Tamil Nadu, Karnataka, where the soils are calcareous in nature. A major impediment in cultivation of groundnut on calcareous and alkaline soils is the occurrence of iron deficiency, which even if abundantly present in the soil system is unavailable to plants as it is present as insoluble iron complexes (Shao *et al*., 2007) [19]. This problem sustains in a worldwide scale where, projected estimations of 30-50% of cultivated soils show a critical deficiency of iron (Cakmak, 2002) [3]. To correct the issue, a major share of traditional chemical fertilizers is used, but difficulties are faced, such as: expensive cost of chelated-Fe fertilizer, adsorption of organic-Fe fertilizer onto soil particles thereby, reducing the fertilizer effect (Lucena *et al*., 2010) [12].

Nanotechnology is a coming-of-age technology that can address the issues related to this agricultural problem. The unique blend of nanotechnology with formulations could give rise to nano-fertilizers that could potentially revolutionize the agricultural sector and aid in enhancement of crop production, disease control and maintaining sustainability in the sector. Nano-fertilizers could be of excellent benefit due to their unique properties such as: precise point of action, surface chemistry, high sensitivity and fast response time (Kumbhakar *et al*., 2014) [9] and could provide a suitable answer to the problems arising by use of economically hazardous traditional fertilizers. Ongoing research are being undertaken to understand the impact of iron oxide nanoparticles on cultivated groundnut on calcareous soils. Thus, the present research aims to highlight the effect of iron oxide nanoparticles on nutritional parameters and yield attributes of groundnut grown in calcareous soils which could thereby reflect the potential benefits of applying iron oxide nanofertilizers to mitigate the iron deficiency found in the soils.

2. Materials and Methods
2.1 Plant Materials and Treatments
The study was carried out as a pot experiment at the Department of Biochemistry, Junagadh Agricultural University and the seeds of groundnut GJG-31 were obtained from Oilseeds Research Station, Junagadh. The experiment was undertaken with five different treatments as follows: T_1: Foliar application with $FeSO_4$, T_2: Control, T_3: Foliar application with iron nanoparticles @ 25 ppm, T_4: Foliar application with iron nanoparticles @ 50 ppm, T_5: Foliar application with iron nanoparticles @ 75 ppm. 5 seeds were sown per pot and the leaves were selected randomly from the plants.

2.2 Nutritional Parameters
2.2.1 Total Protein Content
Groundnut seeds (0.5 g) were crushed in 5 ml of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 6,000 rpm for 15 min. The resulting supernatant was used for protein estimation using the Folin-Cicoltaeu method (Lowry *et al*., 1951) [11]. An aliquot was taken, adjusted to 1 ml with distilled water, and mixed with 5.0 ml of reagent C (a mixture of 2% sodium carbonate in 0.1 N sodium hydroxide and 0.5% copper sulphate with 1% sodium potassium tartrate). After 10 minutes, 0.5 ml of Folin reagent D (diluted 1:1 with distilled water) was added, allowed to stand for 30 minutes for colour development, and then read using UV spectrophotometer at 660 nm.

2.2.2 Total Soluble Sugars
In a boiling tube, 100 milligrams of defatted groundnut powder were measured and subjected to hydrolysis with 5 ml of 2.5 N HCl for three hours, followed by cooling to room temperature. The solution was then neutralized using sodium carbonate until effervescence ceased. After reaching a volume of 100 ml, the mixture was centrifuged, and 0.2 ml of the supernatant was extracted for analysis. This extract was adjusted to 1 ml in volume and mixed with 4 ml of anthrone reagent. The resulting mixture was heated in a boiling water bath for eight minutes, rapidly cooled, and the absorbance was recorded at 630 nm. To determine glucose concentration, a standard curve was generated using various concentrations of a glucose stock solution, and glucose concentration was calculated based on the standard graph (Hedge and Hofreiter, 1962) [6].

2.2.3 Oil Content
The oil content of groundnut kernel was determined according to the A.O.A.C. (1995) guidelines using the Soxhlet method. Four grams of dried groundnut powder were placed in thimbles. Beakers were cleaned, dried at 100 °C, cooled in a desiccator, and weighed to obtain the weight of empty beaker. Each beaker was filled with 80 ml of petroleum ether (boiling point 40-60 °C) and attached to the Soxhlet assembly maintained at 80 °C. Extraction was conducted for 30 minutes near the solvent's boiling point, and the collected fat was placed in a glass beaker. After cooling the beakers in a desiccator for about 5 minutes, they were weighed to determine the final weight. The fat content was calculated as the difference between the initial and final beaker weights and expressed as a percentage.

2.3. Yield Parameters
2.3.1. No. of Pods per Plant
For each plot, we randomly selected five plants, counted the number of pods on each plant, and calculated the average to determine the number of pods per plant for that treatment.

2.3.2. No. of Healthy Pods per Plant
Out of the randomly selected five plants, the healthy number of pods on each plant were calculated and the average value was used to determine the number of healthy pods per plant for the treatment.

2.3.3 Test Weight
Samples of seeds were taken from the harvested produce in each net plot yield, and the weight of 100 seeds (referred to as the test weight) was measured in grams.

2.3.4 Statistical Analysis
The physiological data was analyzed using CRD (Completely Randomized Design) for detection of level of significance among the treatments with different treatments.

3. Results and Discussion
3.1 Nutritional Parameters

Table 1: Effect of iron sources on protein content of groundnut seeds

Treatments	Protein content (%)
T_1 ($FeSO_4$)	23.76
T_2 (Control)	17.84
T_3 (FeO NPs @ 25 ppm)	23.28
T_4 (FeO NPs @ 50 ppm)	25.68
T_5 (FeO NPs @ 75 ppm)	19.34
Mean (T)	21.98
S.Em ±	0.18
CD at 5%	0.53
C.V. %	1.60

The analysis of protein content in groundnut seeds is of paramount importance in understanding how various treatments can influence the nutritional quality of this valuable crop. Table 1 presents a comprehensive overview of these treatments and their respective impacts on protein levels.

The most important finding from this study was the remarkable increase in protein content, which showed 25.68%, when groundnut seeds were treated with iron oxide nanoparticles at a concentration of 50 ppm. This outcome suggests that the application of iron oxide nanoparticles at this specific concentration can significantly enhance the protein content of groundnut seeds.

Following closely in terms of protein content was treatment T_1, where $FeSO_4$ was applied. The seeds in this treatment displayed a substantial protein content of 23.76%. The protein content found from the prior treatment showed that this protein content was on par with the results observed in treatment T_3, where 25 ppm of iron oxide nanoparticles were used, yielding a protein content of 23.28%. These findings suggest that there might be multiple pathways or mechanisms at play in these treatments that lead to increased protein synthesis in the groundnut seeds.

In contrast, treatment T_5, which involved the application of 75 ppm nanoparticles, resulted in a slightly lower protein content of 19.34%. While this figure is still notable and higher than the control group, it shows lesser protein content achieved in the previously mentioned treatments with lower nanoparticle concentrations.

Lastly, the control group, which did not receive any specific treatment, exhibited the lowest protein content at 17.84%. This serves as an essential baseline reference point against which the other treatments can be compared. The fact that even the lowest nanoparticle treatment outperformed the control group depicts the potential of these treatments to enhance the nutritional quality of groundnut seeds.

In conclusion, the study's findings indicate that the application of iron oxide nanoparticles, particularly at 50 ppm, has a substantial and positive effect on increasing the protein content of groundnut seeds. The results observed were in accordance to study conducted by Liu *et al*., 2005 [10], where he found a positive correlation between application of iron nano-carbonate on content of the protein in peanut. Studies conducted by Sheykhbaglou *et al*., 2016 [20] on soybean seeds also showed an increasing trend in effect of nano iron effect on soybean seeds' protein content till application of 0.75g/l iron nano-particles and thereafter, decreasing at 1g/l of nano iron application. Similar effects of positive impact of iron on protein content have also been found in soybean, rapeseed, wheat, safflower as well as in peanut as indicated in the studies of Hemantarajan and Trivedi 1997 [8], Rahman 1992 [17]; Baybordi and Mamedov 2010 [2]; Hemantarajan and Garg 1988 [7]; Ravi *et al*. 2008 [18] and Patel *et al*. 1993; respectively.

Marschner, 1995 [14] suggested that the involvement of iron in several key enzymes of nitrogen fixation could potentially be correlated to its participation in enhancement of protein content.

Table 2: Effect of iron sources on Total Soluble Sugars (TSS) content of groundnut seeds

Treatments	**TSS content (%)**
T_1 ($FeSO_4$)	10.45
T_2 (Control)	8.52
T_3 (FeO NPs @ 25 ppm)	10.00
T_4 (FeO NPs @ 50 ppm)	10.61
T_5 (FeO NPs @ 75 ppm)	9.36
Mean (T)	9.79
S.Em ±	0.14
CD at 5%	0.42
C.V. %	3.06

The evaluation of Total Soluble Sugars (TSS) content in groundnut seeds detailed in Table 2 unravels insights into the relationship between the TSS content and application of iron oxide nanoparticles. The impact of each treatment on TSS content depicts information regarding nutritional value of the groundnut kernels.

Among the findings, the treatment with iron oxide nanoparticles at a concentration of 50 ppm resulted in a substantial increase in TSS content containing 10.61%. Closely following this result, treatment T_1, where $FeSO_4$ was applied, exhibited a TSS content of 10.45%. This parity with the 50 ppm iron oxide nanoparticle treatment suggests that both approaches, despite utilizing different compounds, are comparably effective in elevating TSS content in groundnut seeds. With treatment T_3, the application of 25 ppm iron oxide nanoparticles, produced a TSS content of 10.00%. In contrast, treatment T_5, which employed a higher concentration of 75 ppm nanoparticles, yielded a TSS content of 9.36%. Although this value falls below the results of the lower nanoparticle concentration treatments, it is still higher than the TSS content of the control group. This observation suggests that the positive influence of nanoparticle treatments on TSS content is somewhat concentration-dependent.

The control group, serving as a critical reference point, demonstrated the lowest TSS content at 8.52%. This finding shows significance of the various treatments in elevating the TSS content of groundnut seeds. This is in conformation with the works of El-Metwally *et al*. (2018) [5], where the trend of higher TSS content was observed than the control.

These findings underscore the potential of treatments involving iron oxide nanoparticles and $FeSO_4$ in significantly enhancing the TSS content of groundnut seeds.

Table 3: The effect of iron sources on oil content of groundnut seeds

Treatments	**Oil content (%)**
T_1 ($FeSO_4$)	43.06
T_2 (Control)	41.91
T_3 (FeO NPs @ 25 ppm)	42.45
T_4 (FeO NPs @ 50 ppm)	44.31
T_5 (FeO NPs @ 75 ppm)	42.24
Mean (T)	42.79
S.Em ±	0.31
CD at 5%	0.95
C.V. %	1.47

The assessment of oil content in groundnut seeds across various treatments, as outlined in Table 3.3 signifies the impact of nano iron oxide nanoparticles on the oil content of the seeds from different treatments employed. The highest oil content observed was 44.31%, which was achieved through the application of iron oxide nanoparticles at a concentration of 50 ppm. This result stands out prominently, indicating that this specific treatment has a profound positive influence on enhancing the oil content of groundnut seeds. In the treatment labeled T_1, where $FeSO_4$ was applied, groundnut seeds exhibited an oil content of 43.06%. It was marginally lower than the 50 ppm iron oxide nanoparticle treatment. Treatment T_3, involving the application of 25 ppm iron oxide nanoparticles, yielded an oil content of 42.45%. This is still notably higher than the oil content observed in the control group. Treatment T_5, utilizing a higher concentration of 75 ppm nanoparticles, resulted in an oil content of 42.24%. While this value is somewhat lower than the other treatments mentioned, it still exceeds the oil content observed in the control group showing oil content at 41.91%. This observation suggests that nanoparticle treatments continue to have a beneficial impact on oil content, albeit with some variation based on the concentration applied. The results from this study converges with the results found from Ravi *et al*., 2008 [18], where they have found that application of nano-iron enhances the lipid quantity of safflower. Davar *et al*., 2014 [4], in their study has also found mitigating effects of foliar spray of iron nanoparticles on the oil percentage of safflower.

3.2 Yield Attributes

Table 4: Effect of iron sources on number of pods per plant, number of healthy pods per plant and test weight of groundnut

Treatments	No. of pods per plant	No. of healthy pods per plant	Test weight (g)
T_1 ($FeSO_4$)	17.56	14.14	38.71
T_2 (Control)	12.56	7.85	30.39
T_3 (FeO NPs @ 25 ppm)	15.47	11.88	40.10
T_4 (FeO NPs @ 50 ppm)	19.39	15.12	42.47
T_5 (FeO NPs @ 75 ppm)	13.44	7.94	31.26
Mean (T)	15.68	11.39	36.59
S.Em±	0.25	0.28	0.28
CD at 5%	0.75	0.85	0.85
C.V. %	3.16	4.93	1.54

The data presented in Table 4 provides a comprehensive overview of the effects of various treatments on three crucial parameters related to groundnut yield attributes: the number of pods per plant, the number of healthy pods per plant, and the 100-kernel test weight per plant.

Firstly, when evaluating the number of pods per plant, it is evident that treatment T_4, involving the application of iron oxide nanoparticles at 50 ppm, outperformed other treatments, with an average of 19.39 pods per plant. This indicates that this treatment significantly enhances pod production. Treatment T_1, by application of $FeSO_4$, also yielded impressive results, with an average of 17.56 pods per plant. In contrast, the control treatment (T_2) displayed the lowest pod count, emphasizing the significant difference in pod production between untreated plants and those exposed to various treatments.

Further, the number of healthy pods per plant exhibited similar trends. Treatment T_4, with iron oxide nanoparticles at 50 ppm, demonstrated the highest number of healthy pods, averaging 15.12 per plant. This treatment not only increased pod quantity but also improved their overall health and quality. Treatment T_1, featuring $FeSO_4$ application, showcased a notable average of 14.14 healthy pods per plant. Conversely, the control group (T_2) exhibited the lowest count of healthy pods, emphasizing the stark difference in pod health between treated and untreated plants.

Lastly, the evaluation of the 100 kernel test weight per plant revealed that T_4, with iron oxide nanoparticles at 50 ppm produced the most favorable results, with an average test weight of 42.47 grams per plant. This treatment significantly contributed to kernel weight, indicating its potential to enhance groundnut quality. Treatment T_1, involving $FeSO_4$, displayed the second-best result with an average test weight of 38.71 grams per plant, showcasing its effectiveness in promoting kernel weight. In contrast, the control treatment (T_2) consistently displayed the lowest average test weight, further emphasizing the benefits of applied treatments in improving kernel weight.

In summary, these findings shows the positive impact of specific treatments, especially T_4, on key parameters related to groundnut plant performance, including pod quantity, pod health, and kernel weight. The results are in agreement with reports by Mahmoud *et al*., 2019 where he observed better yield performance when applied with different sources of iron with respect to control.

4. Conclusion

The above study depicted that the foliar spraying with various concentrations of iron nanoparticles shows a significant impact on the increase in nutritional contents and yield attributes of the groundnut kernels and per plant, respectively. The treatment with the application of 50 ppm iron oxide nanoparticles showed favourable result in the above-mentioned parameters with respect to control and other treatments. These results hold promise for optimizing agricultural practices with utilization of nanotechnology to enhance groundnut yield and quality, contributing to food security and improved agricultural productivity.

5. Financial acknowledgement

The author(s) acknowledge the financial assistance provided by UGC (University Grants Commission) in the form of fellowship during the degree programme.

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