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Effect of nano iron oxide on growth and drymatter production of groundnut (*Arachis hypogaea* L.) in a calcareous vertisol

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Abstract
Iron deficiency is a widespread nutritional problem especially in plants growing in high pH and calcareous soils. A green house pot culture experiment was conducted to study the response of groundnut to soil application of nano iron formulations in a calcareous Vertisol. The experiment was conducted in a complete randomized block design with 11 treatments and three replications. Treatments comprised different doses of nano iron oxide in combination with FYM incubation. The results showed that soil application of nano iron oxide enhanced the growth and yield of groundnut. Among all the treatments, Application of nano Fe @ 2.5 mg kg^{-1} mixed with FYM recorded higher plant height (23.5, 26.7 and 35.7 cm), plant spread (23.6 cm, 30.8 cm and 36.7 cm), leaf area $plant^{-1}$ (2.48, 6.42 and 9.95 dm^2) and chlorophyll content (42.5, 48.5, 45.8) at 30, 60 and 90 days respectively. Total dry matter production (58.20 g) was also higher in the same treatment. While, lower growth and dry matter production were observed in control.

Keywords: calcareous soils, groundnut, iron deficiency, nano iron oxide, dry matter production

Introduction
In the Indian oil seed scenario, groundnut (*Arachis hypogaea* L.) is the largest component and occupies 40% of total oilseeds area, 60% of total production (Poonia *et al. 2013*) [10]. Groundnut seed contains 44–50% oil, 26% protein and 10-20% carbohydrates. It is world's largest source of edible oil and ranks 13th among the food crops and 4th most important oil seed crops of the world. Micronutrient deficiency is one of the major constraints in oilseed production which affects the growth, yield and oil quality. Among all the micronutrients, plants require iron more than others (Taiz and Zeiger, 2002) [11]. Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (Brittenham, 1994) [3]. Iron deficiency is a widespread agricultural problem in many crops, especially in groundnut in calcareous and alkaline soils. Calcareous soils may contain high levels of total Fe, but occurs in chemical forms not available to plant roots (Mimmo *et al*., 2014; Bindraban *et al*., 2015) [8, 2]. When active iron (Fe) is low in leaves chlorosis occurs because Fe is required by several enzymes involved in the formation of chlorophyll.

Presently followed methods for treating iron deficiencies were cost ineffective and often give spotty results due to limited penetration. Therefore, efforts need to be made to find out effective remedy to overcome iron stress in crop plants. With the advancement of science, nanotechnology is being visualized as a rapidly evolving field that has potential to revolutionize agriculture production and food systems. Nanoparticles (NPs) are broadly defined as particles having at least one dimension between 1 and 100 nm in diameter (Auffan *et al*. 2009) [1]. With the introduction of high analysis fertilizers and excessive use of chemicals in the past century, we have ended up with polluted soils and underground waters which have been threatening human health and interrupting food chains. The use of nano fertilizer to control release of nutrients can be an effective step towards achieving sustainable agriculture and environment (Cui, 2006) [4]. Reduction of particle size results in increased number of particles per unit of weight and specific surface area of a fertilizer that should increase contact of fertilizer with plant leading to increase in nutrient uptake (Liscano *et al*., 2000) [7]. Thus, nano fertilizers may increase the use efficiency of applied nutrients, enhance yield and nutrient content in the edible parts and also minimize its accumulation in soil and water. In view of the above facts, an attempt was made to study the nano iron nutrition in groundnut growing calcareous Vertisols.

Material and Methods

A green house experiment was conducted at the department of Soil Science & Agricultural chemistry, University of Agricultural Sciences, Dharwad, (Karnataka). The pot culture experiment was conducted in a complete randomized block design with three replications and eleven treatment combinations with groundnut cultivar GPBD- 4.The soil was texturally clay, neutral in pH (7.60), non saline (0.17 dSm^{-1}), medium in organic carbon (5.80 g kg^{-1}), moderately calcareous (9.5%), low in available nitrogen (210 kg N ha^{-1}), medium in available phosphorus (41.6 kg P_2O_5 ha^{-1}) and high in available potassium (475 kg K_2O ha^{-1}). The soil was deficient in iron (4.13 mg kg^{-1}) and zinc (0.44 mg kg^{-1}) and sufficient in Cu (1.61 mg kg^{-1}) and Mn (5.50 mg kg^{-1}). Treatments comprised of different doses of nano iron oxide in combination with FYM incubation. Treatments include T_1 : Absolute control (No RDF), T_2 : control I (Recommended package of practices Includes FYM @ 7.5 t + 25 kg N +50 kg P_2O_5 + 25 kg K_2O + 500 kg Gypsum + 25 kg $ZnSO_4$. $7H_2O$ + 25 kg $FeSO_4.7H_2O$ ha^{-1}), T_3 : control II (Recommended package of practices as in control I but $FeSO_4$ is chelated with FYM in the ratio 1:100 and incubated for 7 days and applied to soil), From treatments T_4 to T_{11} RPP is common and changes has been done in iron doses and mode of application. T_4 : Nano Fe @ 2.5 mg kg^{-1} (Supplies iron equivalalent to Recommended dose of $FeSO_4$ i.e, 25kg /ha), T_5: Nano Fe @ 2.5 mg kg^{-1} mixed with FYM (1 : 100), T_6 : Nano Fe @ 1.25 mg kg^{-1}, T_7: Nano Fe @ 1.25 mg kg^{-1} mixed with FYM (1: 100), T_8 : Nano Fe @ 0.5 mg kg^{-1}, T_9: Nano Fe @ 0.5 mg kg^{-1} mixing with FYM (1: 100), T_{10} : Nano Fe @ 0.25 mg kg^{-1}, T_{11}: Nano Fe @ 0.25 mg kg^{-1} mixing with FYM (1: 100). Nano Fe_2O_3 (<50 nm size) particles, purchased from Sigma Aldrich company was used. The morphology and particle size of nano iron oxides were confirmed by UV-visible spectrophotometer, field emission scanning electron microscope (FE-SEM) and X ray diffraction pattern. Examination of the peaks and image obtained from SEM revealed that the particles are cubic, highly uniform in nature and having average size ranged from 20 - 45 nm. The entire quantity of nitrogen, phosphorus and potassium and zinc were applied to all the treatments at the time of sowing except treatment T_1. Iron was applied in the form of $FeSO_4.7H_2O$ to treatment T_2. Iron sulphate and different sized nano iron oxides were mixed with FYM and cured for 7 days and then incorporated into soil according to treatment details. Initial soil sample anlaysis was carried out by adopting standard methods. Morphological observations were recorded at different growth stages of crop. Chlorophyll content was measured with SPAD meter.

Results and Discussion

The plant height, plant spread of groundnut was significantly influenced by soil application of nano iron oxide formulations at different growth stages (Table 1). At 30, 60 and 90 DAS, application of nano Fe @ 2.5 mg kg^{-1} mixed with FYM (1: 100) recorded significantly higher plant height (23.50, 26.73 and 35.70 cm), Plant spread (23.67 cm, 30.83 cm and 36.70 cm) which was superior to all other treatments. The treatment T_4 which received nano Fe @ 2.5 mg kg^{-1} was on par with T_5. Whereas, the lowest plant height and plant spread was recorded with absolute control. This may be due to slow release of nano iron from the FYM because of chelation. Wahab (2008) [13] reported that iron has important roles in plant growth and yield of plants. Micronutrients, especially Fe acts as metal components of various enzymes and also associated with saccharide metabolism, photosynthesis and protein synthesis.

There is a significant difference among the treatments with respect to leaf area and chlorophyll content. At 30, 60 and 90 DAS significantly higher leaf area (2.48, 6.42 and 9.95 dm^2) was recorded by soil application of nano Fe @ 2.5 mg kg^{-1} mixed with FYM at 1: 100 ratio (T_5). However T_4 (2.32 dm^2) and T_7 (2.28 dm^2) were on par with T_5 at 30 DAS. Lowest leaf area was observed in absolute control (T_1: 1.84, 5.40 and 8.99 dm^2, respectively at 30, 60 and 90 DAS). Reduction in particle size results in increased number of particles per unit of weight and specific surface area of a fertilizer that will increase contact of fertilizer with FYM leading to increased chelation compared to $FeSO_4$. Similar results were reported for interaction of nano fertilizers with plants by Liscano *et al.* (2000) [7]. Kakiuchi and Kobata (2008) [6] reported that microelements affect leaf area which led to larger amounts of assimilate production in common bean.

The chlorophyll content of leaf differed significantly due to the soil application of nano iron oxide formulations (Table 2) at all the crop growth stages i.e., 30, 60 and 90 DAS. Treatment (T_5) receiving nano Fe @ 2.5 mg kg^{-1} mixed with FYM recorded significantly higher chlorophyll content at 30 DAS (42.5), 60 DAS (48.5) and 90 DAS (45.8) Compared to T_2 and T_3 which received RPP and RPP with Chelated FeSO4. $7H_2O$. Absolute control treatment (T_1) registered lowest chlorophyll content at all stages (34.4, 39.4 and 37.0 at 30, 60 and 90 DAS, respectively). Nano-particles of iron oxide exhibit higher surface area which enhances complexation with FYM resulting in an increase in Fe availability for plants (Nia *et al*., 2010) [9]. The increase in chlorophyll content is undoubtedly a result of the function of iron as a stimulator of the activity of chlorophyll synthesis enzymes. Studies indicated that iron functions in the synthesis of a specific kind of RNA that in turn regulates chlorophyll synthesis through a chain of unknown reactions (Jia *et al*., 2012) [5]. Iron is a structural component of porphyrin molecules: cytochromes, hemes, hematin, ferrichrome and leghemoglobin. These substances are involved in oxidation reduction reaction during respiration and photosynthesis which in turn leads to higher chlorophyll content. Yadav *et al*. (2007) [14] also reported that the higher availability of both macro- and micro-nutrients in soil due to combined application of FYM + pyrite which led to increased photosynthesis, resulting in increased production of assimilates.

The total dry matter $plant^{-1}$ production differed significantly due to soil application of nano iron oxide formulations at 60 DAS and at harvest (Table 3). The treatment (T_5) received nano Fe @ 2.5 mg kg^{-1} mixed with FYM at 1: 100 ratio recorded significantly higher TDMP at 60 DAS (24.43 g $plant^{-1}$) and at harvest (58.20 g $plant^{-1}$). Higher dry matter accumulation in plant was contributed by Pods followed by leaf and stem dry weight. At 60 DAS and harvest, highest stem (8.70 and 15.60 g $plant^{-1}$), leaf (7.56 and 16.70g $plant^{-1}$) and pod (8.17 and 24.23 g $plant^{-1}$) dry weight was observed in the treatment (T_5), which received soil application of @ 2.5 mg kg^{-1} mixed with FYM at 1: 100 which is significantly superior to T_2 and T_3. The Lowest DM accumulation in stem (5.32 and 7.34 g $plant^{-1}$), leaf (4.71 and 9.23 g $plant^{-1}$) and pod (7.25 and 12.40 g $plant^{-1}$) were observed in absolute control (T_1). Enhanced dry matter production might be due to improvement in nutrient uptake particularly iron and zinc along with nitrogen and phosphorus which favourably influence carbohydrate metabolism and increased transformation of photosynthetic activity towards growing plant parts. Similar findings were observed by Thomas and Thenua (2010) [12]. The increased dry matter may also be due to beneficial effect of FYM in conjunction with Iron. Treatments received nano iron chelated with FYM was recorded higher dry matter production compared to their respective unchelated counterparts. This is due to unchelated Fe was precipitated as its carbonate which is not available for the crop growth.

Table 1: Plant height and plant spread of groundnut at different growth stages as influenced by soil application of nano iron oxide formulations

Treatment details	Plant height (cm)			Plant spread (cm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1 : Absolute control	14.7 g	18.5 e	26.6 g	16.1 g	22.6 g	25.1 g
T_2 : Control I (RPP)	17.7 de	21.8 bc	31.2 d	19.7 c-e	26.6 de	29.7 d
T_3: Control II (RPP ($FeSO_4$ mixed with FYM 1 : 100))	19.7 bc	23.1 b	32.8 b-d	20.6 c	28.1 cd	32.1 c
T_4 : Nano Fe @ 2.5 mg kg^{-1} (5 kg Fe ha^{-1})	21.9 a	25.7 a	34.2 ab	22.7 ab	29.8 ab	33.8 b
T_5: Nano Fe @ 2.5 mg kg^{-1} mixed with FYM (1 : 100)	23.5 a	26.7 a	35.7 a	23.6 a	30.8 a	36.7 a
T_6 : Nano Fe @ 1.25 mg kg^{-1}	18.4 cd	22.5 bc	31.8 cd	20.1 cd	27.6cd	31.5 c
T_7 : Nano Fe @ 1.25 mg kg^{-1} mixed with FYM (1: 100)	20.1 b	23.4 b	33.2 bc	21.9 b	29.1 bc	32.6 bc
T_8 : Nano Fe @ 0.5 mg kg^{-1}	16.2 e-g	20.9 cd	28.6 ef	18.9 d-f	24.8 f	28.3 d-f
T_9: Nano Fe @ 0.5 mg kg^{-1} mixing with FYM (1: 100)	17.3 d-f	21.2 cd	29.3 e	19.4 c-f	25.5 ef	29.2 de
T_{10} : Nano Fe @ 0.25 mg kg^{-1}	15.2 g	19.7 de	27.4 fg	18.1f	24.4 f	26.8 f
T_{11}: Nano Fe @ 0.25 mg kg^{-1} mixing with FYM (1: 100)	15.8 fg	20.1 d	28.2 e-g	18.4 ef	24.6 f	27.9 ef
S.Em. $\pm$	0.52	0.52	0.56	0.42	0.53	0.56

Note

1. RPP – FYM @ 7.5 t + 25 kg N +50 kg P_2O_5 + 25 kg K_2O + 500 kg Gypsum + 25 kg $ZnSO_4$. $7H_2O$ + 25 kg $FeSO_4.7H_2O$ ha^{-1}
2. Treatments T_4 to T_{11} RPP is common except soil application of $FeSO_4.7H_2O$.
3. DAS-Days after sowing

Table 2: Leaf area and chlorophyll content of groundnut at different growth stages as influenced by soil application of nano iron oxide formulations

Treatment details	Leaf area (dm^2)			Chlorophyll content of leaf (spad values)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1: Absolute control	1.84 g	5.40 h	8.99 f	34.4 g	39.4 h	37.0 h
T_2: Control I (RPP)	2.12 b-f	5.89 c-f	9.52 b-e	38.2 c-e	44.5 d-f	41.5 d-f
T_3: Control II (RPP ($FeSO_4$ mixed with FYM 1 : 100))	2.21 b-d	6.01 b-d	9.61 b-d	39.6 bc	45.6 b-d	42.6 b-d
T_4 : Nano Fe @ 2.5 mg kg^{-1} (5 kg Fe ha^{-1})	2.32 ab	6.20 ab	9.71 ab	40.7 b	47.1 ab	44.1 ab
T_5: Nano Fe @ 2.5 mg kg^{-1} mixed with FYM (1 : 100)	2.48 a	6.42 a	9.95 a	42.5 a	48.5 a	45.8 a
T_6 : Nano Fe @ 1.25 mg kg^{-1}	2.18 b-e	5.94 c-e	9.56 b-d	39.1 b-d	45.1 c-e	42.1 c-e
T_7: Nano Fe @ 1.25 mg kg^{-1} mixed with FYM (1: 100)	2.28 a-c	6.09 bc	9.65 bc	40.8 b	46.7 b c	43.7 bc
T_8 : Nano Fe @ 0.5 mg kg^{-1}	2.02 d-g	5.73 e-g	9.36 de	37.1 ef	43.1 fg	40.1 fg
T_9: Nano Fe @ 0.5 mg kg^{-1} mixing with FYM (1: 100)	2.08 c-f	5.81 d-g	9.43 c-e	37.7 d-f	43.7 e-g	40.7 e-g
T_{10} : Nano Fe @ 0.25 mg kg^{-1}	1.95 fg	5.64 g	9.27 e	36.2 f	42.2 g	39.2 g
T_{11}: Nano Fe @ 0.25 mg kg^{-1} mixing with FYM (1: 100)	1.98 e-g	5.69 f-g	9.28 e	36.7 ef	42.7 g	39.7 fg
S.Em. $\pm$	0.07	0.08	0.09	0.56	0.57	0.60

Note

1. RPP – FYM @ 7.5 t + 25 kg N +50 kg P_2O_5 + 25 kg K_2O + 500 kg Gypsum + 25 kg $ZnSO_4$. $7H_2O$ + 25 kg $FeSO_4.7H_2O$ ha^{-1}
2. Treatments T_4 to T_{11} RPP is common except soil application of $FeSO_4.7H_2O$.
3. DAS-Days after sowing

Table 3: Dry matter accumulation and total dry matter production (TDMP) of groundnut at different growth stages as influenced by soil application of nano iron oxide formulations

Treatment details	Dry matter production (g $plant^{-1}$)															
	60 DAS								Harvest							
	Stem		Leaf		Pod		TDMP		Stem		Leaf		Pod		TDMP	
T_1: Absolute control	5.32	h	4.71	h	5.75	i	15.7	j	7.34	i	9.23	g	13.2	i	29.7	i
T_2: Control I (RPP)	7.12	b-f	5.67	d-f	7.05	de	19.8	ef	10.6	ef	11.8	de	17.2	d-f	39.6	ef
T_3: Control II (RPP ($FeSO_4$ mixed with FYM 1 : 100))	7.52	b-d	6.05	cd	7.49	bc	21.0	cd	11.9	cd	13.5	c	18.6	cd	44.0	d
T_4: Nano Fe @ 2.5 mg kg^{-1} (5 kg Fe ha^{-1})	8.06	b	6.87	b	7.81	b	22.7	b	13.8	b	15.4	b	22.6	b	51.8	b
T_5: Nano Fe @ 2.5 mg kg^{-1} mixed with FYM (1 : 100)	8.70	a	7.56	a	8.17	a	24.4	a	15.6	a	16.7	a	24.2	a	58.2	a
T_6: Nano Fe @ 1.25 mg kg^{-1}	7.34	bc	5.89	c-e	7.25	cd	20.4	de	11.1	de	12.4	d	18.0	de	41.5	e
T_7: Nano Fe @ 1.25 mg kg^{-1} mixed with FYM (1: 100)	7.82	bc	6.22	c	7.68	b	21.7	c	12.6	c	14.6	b	19.7	c	46.9	c
T_8: Nano Fe @ 0.5 mg kg^{-1}	6.51	fg	5.32	fg	6.61	fg	18.4	gh	9.10	gh	10.7	f	15.9	f-h	35.7	gh
T_9: Nano Fe @ 0.5 mg kg^{-1} mixing with FYM (1: 100)	6.84	ef	5.45	e-g	6.86	ef	19.1	fg	9.85	fg	11.1	ef	16.6	e-g	37.5	fg
T_{10} : Nano Fe @ 0.25 mg kg^{-1}	6.01	g	5.19	g	6.17	h	17.3	i	8.57	h	10.4	f	14.8	h	33.8	h
T_{11}: Nano Fe @ 0.25 mg kg^{-1} mixing with FYM (1: 100)	6.22	g	5.29	fg	6.45	gh	17.9	hi	8.77	h	10.5	f	15.2	gh	34.5	h
S.Em. $\pm$	0.20		0.15		0.12		0.35		0.28		0.35		0.47		0.79	

Note

1. RPP – FYM @ 7.5 t + 25 kg N +50 kg P_2O_5 + 25 kg K_2O + 500 kg Gypsum + 25 kg $ZnSO_4$. $7H_2O$ + 25 kg $FeSO_4.7H_2O$ ha^{-1}
2. Treatments T_4 to T_{11} RPP is common except soil application of $FeSO_4.7H_2O$.
3. DAS-Days after sowing.

Conclusion

Soil application of nano iron oxide formulations had a significant effect on growth parameters at all stages of groundnut. Application of nano Fe @ 2.5 mg kg^{-1} mixed with FYM recorded higher growth parameters of groundnut. With the increase in quantity of nano iron added to soil, higher the growth and yield parameters were observed in groundnut crop.

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