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D Kanjana
Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Correspondence
D Kanjana
Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Foliar study on effect of iron oxide nanoparticles as an alternate source of iron fertilizer to cotton

D Kanjana

Abstract
An experiment was carried out under pot culture to investigate the effects of different iron sources of fertilizers like iron sulphate, iron oxide and nano sized iron oxide particles *viz.,* haematite and magnetite with different levels of concentration viz., 2, 4, 6, 8 and 10 g/lit on cotton growth, development and yield of cotton. Treatments were replicated into three and foliar sprayed (20 ml per pot) on square formation (45 DAS) and boll formation (90 DAS) stages. The study showed that significant increment of plant height (37 cm), leaf area (45.4 cm^2/plant), number of symbodial branches per plant (15.1), seed cotton yield (14.9 g/pot) and boll weight (3.5 g/boll) were attained due to foliar application of magnetite nanoparticles during both square and flower formation stages. It is concluded that oxide type iron nanoparticles particularly magnetite was superior than normal iron oxide on increasing plant growth parameters as well as seed cotton yield but it was onpar with sulphate type fertilizers.

Keywords: Iron fertilizers, magnetite, haematite, cotton, iron oxide nanoparticles nanotechnology

1. Introduction

Iron is the fourth most abundant element in the earth's crust after oxygen, silicon and aluminium (Ma, 2005) [20] but it is unique with regard to its solubility in soils and its availability to crops which are governed by soil pH (Ryan *et al.* 2013) [26]. Iron deficiency symptom *ie.,* iron chlorosis is a common nutrient disorder in plants grown in high pH and calcareous soils which results in poor yield and quality of the crops due to decreased leaf photosynthetic pigment concentrations, especially chlorophyll (Chen *et al.* 2016) [8]. Cotton is an important cash crop in India and is generally grown in heavy clay soils with high pH, where the micronutrient availability particularly iron is reduced and this leads to development of iron deficiency which affects growth, development and productivity of cotton (Fageria *et al.* 1990 [11] & Eleyan *et al. 2014*) [9]. As compared to soil application, foliar feeding is an effective way to increase the iron in leaves when the roots cannot provide the necessary nutrients to the reproductive parts of the plants like soybean (Alidoust & Isoda, 2013) [2]. The generally used soluble form of iron sulphate fertilizers contains only 20 % of Fe whereas the insoluble form of iron oxide contains 70 % Fe. Due to the technological advancement *ie.,* nanotechnology, the insoluble metal iron oxide particle can be converted into soluble form by reducing their particle size and increasing their solubility rate through physical, chemical and biological methods and hence higher nutrient content of iron oxide can be highly utilized for increasing the iron concentration and yield of plants as compared to sulphate form fertilizers (Kanjana, 2017) [15]. Recently, iron oxide nanoparticles (Fe_2O_3/Fe_3O_4 NP) have been widely applied in catalytic reactions, magnetic field, biomedicine, degradation of dye, removal of heavy metals, waste water treatment and other fields (Saif *et al.* 2016) [27]. Due to its unique properties of smaller size, larger surface area and higher reactivity, this new type of fertilizer *ie.,* iron oxide nanoparticles are being highly used to study their effects on various crop plants. Most of the studies reported the positive effects of Haematite (Fe_2O_3) NPs on various crop plants like peanut (Rui *et al.* 2016) [25], soybean (Sheykhbaglou *et al.* 2010) [29] and wheat (Ghafari & Razmjoo, 2013) [12]. But some of the studies showed the negative effects of iron oxide nanoparticle particularly magnetite (Fe_3O_4) on pumpkin plants (Zhu *et al.* 2008) [32]. Inversely, Elfeky *et al.* (2013) [10] studied the positive effects of magnetite nanoparticles (Fe_3O_4) on sweet basil. However, research on effectiveness of insoluble form of metal oxide and their nanoparticles has not been well studied on crop plants particularly on cotton. Therefore, the objective of the paper was to evaluate the potentiality of metal oxide nanoparticles (Fe_2O_3 and Fe_3O_4) along with bulk metal oxide (FeO) and conventional iron sulphate ($FeSO_4$) fertilizers on growth and yield of cotton crop.

2. Materials and Methods

In order to study the effects of foliar application of different levels of normal and nano iron oxide fertilizers on cotton growth and yield, an experiment was carried out at Regional Station, Central Institute for Cotton Research, Coimbatore under pot culture. The experimental design was laid out in factorial based randomized complete block design (FCRD). Pots, 35 cm height and 33 cm internal diameter, were used for this study. Surface soil (0–20 cm) was collected from fallow land of CICR Research farm. The collected soil samples were analyzed for physical, physico chemical and chemical properties by standard soil testing procedures given by Baruah & Barthakur (1999) [5] and presented in Table 1.

Table 1: Characteristics of initial soil samples used for pot culture study

S. No.	Parameters	Value
1.	Soil Texture	Clay loam
2.	Soil reaction (1:2.5 soil water suspension)	8.6
3.	Electrical conductivity (dS/m) (1:2.5 soil water extract)	0.30
4.	Cation exchange capacity (cmol (p^+)/kg soil)	20.4
5.	Organic carbon (%)	0.45
6.	Available Nitrogen (kg/ha)	106.5
7.	Available Phosphorus (kg/ha)	10.5
8.	Available Potassium (kg/ha)	758
	DTPA extractable micronutrients (mg/kg)	
9.	Zinc	0.48
10.	Iron	2.68
11.	Copper	1.22
12.	Manganese	7.2

Approximately, 25 kg of mixed soil samples were used to fill each pot. Cotton (*Gossipium hirsutum*) variety suraj seeds (4 nos.) were sown at 3 cm depth manually in each pot which were thinned to one per pot after seedling establishment. For all the treatments including control, recommended dose of fertilizer (60:30:30 kg NPK ha^{-1}) was applied uniformly, in which P as single super phosphate (2.2 g/pot) and K as muriate of potash (0.6 g/pot) were applied as basal and half dose of N as urea (0.8 g/pot) was applied at 15 DAS and remaining half dose of N (0.8 g/pot) was applied at 85-90 DAS. During the year 2014-15 winter irrigated seasons (August – February), five treatments *viz.*, iron sources of fertilizers like F1 – Iron sulphate ($FeSO_4$), F2 - Iron oxide (FeO), F3 - Nano iron oxide – haematite form (Fe_2O_3), F4 - Nano iron oxide – magnetite form (Fe_3O_4) and F5 – Control with different concentrations of 2, 4, 6, 8 and 10 g/lit were replicated into three and these treatments were foliar sprayed (20 ml per pot) at each stages of square formation (45 DAS) and boll formation (90 DAS). Iron III oxide – (FeO <5µm), Iron III oxide nano powder (Fe_2O_3 <50 nm) and Iron II, III oxide (Fe_3O_4 50-100 nm) were purchased from Sigma-Aldrich company, USA but Ferrous sulphate heptahydtrate ($FeSO_4 7H_2O$) was procured from Himedia Lab, Mumbai. Iron oxide (F2) and iron oxide nanoparticles (F3 and F4) were weighed separately and suspended directly in deionized water to disperse the particles using probe sonicator (100 W, 40 khz) for 10 min.

Plant height (soil surface to plant top) and chlorophyll content in 4th fully expanded leaf (SPAD -502 plus chlorophyll meter – Konica-Minolta, Japan) were measured at 15 days after first foliar spray. Likewise, 15 days after second spray, plant height, number of leaves and leaf area were recorded. Leaf area was calculated by multiplying the product of leaf length and width by the factor 0.77 for *G. hirsutum* (Ashley *et al.* 1963 [4] & Su *et al.* 2015 [30]). At harvest stage of the crop, yield related parameters *viz.*, number of symbodial branches per plant, number of total bolls per plant, seed cotton yield and boll weight were recorded. For calculating the dry matter, shoot and root were separately cut and their dry weight was recorded after keeping the samples in an oven at 65ºC. Finally data was statistically analyzed using standard analysis of variance.

3. Results and Discussion

3.1. Growth parameter

Results of Analysis of Variance (ANOVA) showed that plant height at flowering stage (60 DAS) and leaf area at boll formation stage (105 DAS) were significantly affected due to different sources of iron fertilizers (F) ($p \leq 0.05$) and interaction of different sources of iron and their levels (FxD) respectively. The other growth parameters like plant height and number of leaves at boll formation stage (105 DAS) were not significantly affected by both different sources (F) and their levels (D) of iron fertilizers (Table 2). The highest plant height (37 and 52 cm) was recorded in iron oxide (magnetite, Fe_3O_4) nanoparticles and the lowest (28.3 and 41.5 cm) was registered in control treatment at various stages *viz.*, flowering and boll formation stage. This result was same with the findings of Salarpour *et al.* (2013) [28], and they reported that the highest plant height (32.72 cm) of cress *(Lepidium sativum* L.) was obtained using 5g nano iron chelate + foliar spraying that was increased by 80% compared with control treatment. Bozorgi (2012) [7] reported that the lowest plant height of eggplant, with 68.83 cm was found in without nano iron chelate and nitrogen fertilizer treatment. These results confirmed that nanosized iron particles highly influenced on improving the plant height character. Abdallah & Mohamed (2013) [1] showed that cotton cultivars significantly varied in plant height due to Mn, Fe and Zn application. Iron acts as a cofactor for approximately more than hundred enzymes and being a component of ferrodoxin, an electron transport protein associated with chloroplast might have helped to increase the growth characters of plants (Hazra *et al.* 1987) [13]. Application of 4 g/lit of different sources of iron fertilizers had maximum effect on plant height at 60 and 105 DAS but it was significantly indifferent.

Table 2: Analysis of variance of growth and physiological parameters of cotton at flowering and boll formation stages

Sources of variation	Degrees of freedom	Plant height, cm	Plant height, cm	No. of Leaves	Leaf area cm^2/ plant
		60 DAS	105 DAS		
Sources of iron fertilizers (F)	4	204.4*	252.7 ns	133.4 ns	50.6 ns
Doses of fertilizer (D)	4	103.2 ns	139.8 ns	17.8 ns	83.6 ns
F x D	16	87.8 ns	115.9 ns	20.3 ns	420.5 **
Error	48	45.0	103.0	53.5	177.4
Coefficient of Variation (%)		15.8	18.7	28.3	29.1

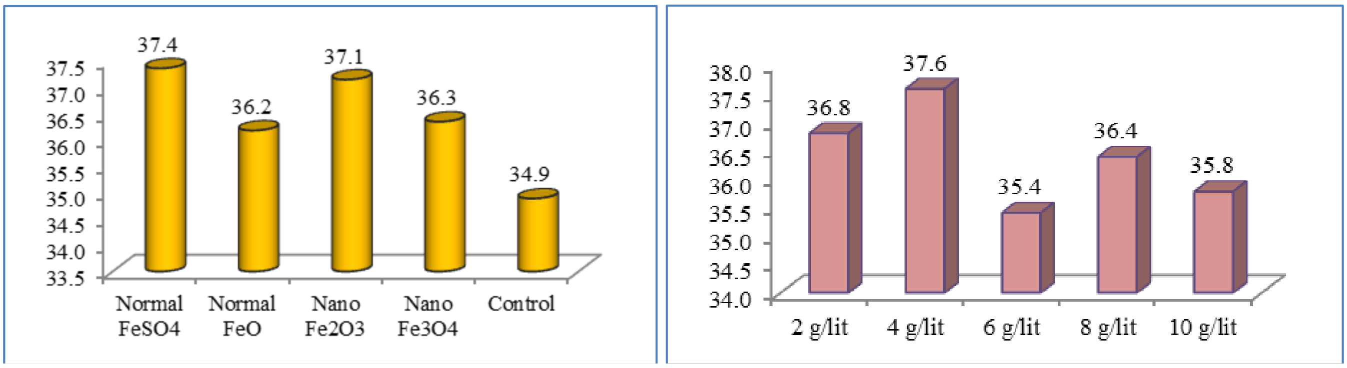
3.2. Physiological parameter

Regarding the leaf characters of cotton, higher number of leaves (28.9) was produced in iron oxide (magnetite, Fe_3O_4) nanoparticles and lower number of leaves was noted in normal iron oxide (20.8) and control (25.4) treatment. The highest leaf area (45.4 cm^2/plant) was recorded in iron oxide (magnetite, Fe_3O_4) nanoparticles and the lowest (41.9 cm^2/plant) was registered in control. Next to magnetite nanoparticles, all the above mentioned parameters were increased in normal iron sulphate ($FeSO_4$) followed by nano iron oxide haematite (Fe_2O_3) and normal iron oxide (FeO) fertilizers (Table 3). Pirzad & Shokrani (2012) [24] reported that leaf characters like number of leaves, leaf weight and leaf area of marigold (*Calendula officinalis L.)* were significantly increased by 1.5 l/ha of nano iron chelate (Khazra Company). Similarly Mamyandi *et al.* (2012) [21] found the increased leaf length and leaf width in sugarbeet due to foliar application of nano-iron chelate.

Table 3: Mean comparison of growth and physiological parameters of cotton at flowering and boll formation stages

Treatments		Plant height cm	Plant height cm	No. of Leaves	Leaf area cm^2/ plant
		60 DAS	105 DAS		
Iron sources of fertilizers (F)					
Normal $FeSO_4$	F1	36.0	50.4	26.8	44.9
Normal FeO	F2	35.8	46.7	20.8	41.2
Nano Fe_2O_3	F3	31.5	46.1	25.5	42.8
Nano Fe_3O_4	F4	37.0	52.0	28.9	45.4
Control	F5	28.3	41.5	25.4	41.9
Levels of concentration (D)					
2 g/lit	D1	29.8	43.3	24.5	42.1
4 g/lit	D2	35.1	51.5	27.1	47.3
6 g/lit	D3	35.5	47.6	25.9	42.2
8 g/lit	D4	35.8	48.5	24.4	41.5
10 g/lit	D5	32.2	45.9	25.6	43.0

The next physiological attribute is chlorophyll content of the leaves, which is an important index of plant growth. SPAD chlorophyll value showed higher chlorophyll content in normal iron sulphate fertilizer (37.4) which is equal with haematite nanoparticle (37.1) followed by magnetite nanoparticles (36.3) and normal iron oxide (36.2), whereas the lower chlorophyll content was in control (34.9) treatment (Fig.1A). This result indicated that iron acts as a stimulator of activity of chlorophyll synthesis enzyme. Apart from this, iron involves in various plant components such as catalase, ferredoxin, ferrichrome, hematin, heme, cytochrome oxidase and nucleic acid metabolism (Venu *et al.* 2016) [31]. Although soluble $FeSO_4$ fertilizers notably increased the chlorophyll value due to active participation of both iron and sulphur nutrient besides, immediate availability of iron to the mesophyll cells of leaf and decreased chlorophyll degradation caused by stress (Kotyal *et al.* 2017) [16] but the insoluble iron oxide nanoparticles performance was much better at low concentration on increasing the chlorophyll content in cotton leaves which may be due to precipitation of iron oxide nanopartilcles in the leaves after foliar spray as insoluble oxides forming complexes with phytoferritin, an iron-binding protein found in leaves. Consequently, low mobility of iron after binding to phytoferritin in leaves leads to higher contribution in chlorophyll synthesis (Bienfait & Der Mark, 1983) [6]. Jalali & Zargani (2014) [14] resulted that the highest total chlorophyll content (14.14 mg/g) in lettuce leaves was recorded by application of nano Fe-chelate 9% and the lowest (6.43 mg/g) in $FeSO_4$ treatment under NFT hydroponics system. Even though there is no direct role of Fe in chlorophyll structure, but enough Fe improves chlorophyll in plants and plant chlorophyll status that can affect the rate of photosynthesis (Peyvandi *et al.* 2010) [23]. Among the different levels of iron source of fertilizers, 4g/lit of iron concentration of various iron sources of fertilizers invariably increases the physiological parameters *viz.*, number of leaves, leaf area (Table 3) and chlorophyll SPAD value (Fig.1B) on cotton but it was not significant.



(a) **(b)**

Fig 1: Effect of different sources of iron fertilizers (A) and different levels of iron fertilizers (B) on Chlorophyll (SPAD) value

3.3. Yield parameters

Results of analysis of variance (ANOVA) showed the significant effect of iron sources of fertilizers on yield parameters *viz.*, number of symbodial branches per plant, seed cotton yield and boll weight. The other yield parameters like number of total bolls per plant and number of opened bolls per plant were not significantly affected by both iron sources as well as levels of iron fertilizers (Table 4). Mean comparison indicated that number of symbodial branches per plant was increased by iron oxide nanoparticles *ie.*, magnetite (15.1) followed by haematite (13.7) as compared to normal iron sulphate (12.5) and iron oxide fertilizers (12.4) respectively. This is the most reliable parameter on increasing the seed cotton yield. Similar result was obtained by Eleyan *et al.* (2014) [9] who reported 18.6 % of number of sympodial branches per plant (22.1) was registered in cotton due to foliar application of iron sulphate @ 200 mg/lit. The increased iron in leaves might have increased the production of metabolites and thus the plant had the chance to bear more fruiting branches. Similarly, boll weight is also one of the important factors to increase the seed cotton yield. The highest boll weight was recorded in application of magnetite (3.5 g/boll) followed by iron sulphate (3.3) and nano iron oxide, haematite (3.0) and the lowest was obtained in control (2.6 g/boll). This was in line with the agreement of Sheykhbaglou *et al.* (2010) [29] who found that leaf and pod dry weight of soybean was significantly increased by application of 0.75 g/lit concentration of nano iron oxide.

Regarding the number of total bolls per plant, magnetite nanoparticles increased this parameter as like other growth, physiological and yield parameters but the number of opened bolls per plant was not markedly increased but it was onpar with all the other iron sources of fertilizers. These results confirmed that the above mentioned yield related parameters contributing significant increase in seed cotton yield under technologically advanced iron oxide fertilizers *ie.*, magnetite nanoparticles, but it was onpar with generally used iron sulphate fertilizers. The highest seed cotton yield (14.9 and 14.8 g/pot) was recorded by both nano Fe_3O_4 and normal $FeSO_4$ fertilizers respectively whereas the lowest seed cotton yield (10.5 g/pot) was recorded in control treatment (Table 5). This result was more similar with the findings of Sheykhbaglou *et al.* (2010) [29] who reported that 48 % of increased grain yield in soybean was recorded in 0.5 g/lit of iron oxide nanoparticles than control. Elfeky *et al.* (2013) [10] found that foliar application of Fe_3O_4 (magnetite) NPs on sweet basil plants significantly increased total chlorophyll content, total carbohydrate, iron content, plant height, number of leaves/plant and number of branches/plant, fresh weight and dry weight especially at concentrations of 3 mg/L.

Iron is an essentially required microelement for photosynthetic carbon acquisition, synthesis of nitrate reductase, photosynthetic electron transport systems (iron in pheophytin, ferredoxin and cytochromes) and functions as a cofactor for a variety of enzymes, such as iron in coproporphyrinogen III oxidase functioning in chlorophyll biosynthesis pathway. Therefore, this much importance of iron could be efficiently utilized through advanced technology *ie.*, nanotechnology where the particles are nanosized (10^{-9}m) with unique properties of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. Among the various levels of iron sources of fertilizers *viz.*, 2, 4, 6, 8 and 10 g/ lit, the maximum level of yield related parameters like number of symbodial branches per plant, number of total bolls per plant, seed cotton yield and boll weight was obtained in ideal concentration of 4g/lit of iron sources of fertilizers, but it was no significant. Similarly, no significant variation was attained between iron sources of fertilizers and their levels on yield related parameters of cotton.

Table 4: Analysis of variance of yield related parameters of cotton at harvest stage

Sources of variation	Degrees of freedom	No. of symbodial branches/ plant	No. of total bolls / plant	No. of opened bolls / plant	Seed cotton yield (g/pot)	Boll wt (g/ boll)	Shoot dry weight (g/ plant)	Root dry weight (g/ plant)
Sources of iron fertilizers (F)	4	22.5 *	72.1 ns	0.95 ns	46.8*	1.75 *	230.4 ns	4.39 **
Doses of fertilizer (D)	4	14.5 ns	16.7 ns	1.05 ns	6.6 ns	0.06 ns	266.2 ns	0.32 ns
F x D	16	9.6 ns	38.9 ns	0.91 ns	6.4 ns	0.32 ns	138.8 ns	1.27 ns
Error	48	7.0	29.3	0.72	12.9	0.52	99.1	0.89
Coefficient of Variation (%)		20.3	41.5	18.2	27.4	25.0	28.6	27.1

3.4. Dry matter production

According to the analysis of variance in Table 4, shoot dry weight of cotton was not significantly increased by various sources (F) and levels (D) of iron fertilizer but in converse, root dry weight of cotton was significantly improved by different iron sources of fertilizers as compared to various levels of iron fertilizers and their interactions. Shoot dry weight of cotton was higher in iron oxide nanoparticles like magnetite (40.5 g/plant) and haematite (36.6 g/plant) followed by iron sulphate (33.2 g/plant) fertilizers. But root weight of cotton was significantly increased by application of iron oxide nanoparticles like haematite (4.5 g/plant) followed by magnetite (3.8 g/plant) as compared to control treatment (3.1 g/plant). Some of the researchers reported that iron oxide nanoparticles particularly Fe_2O_3 produced significant positive effect on root elongation on watermelon (Li *et al.* 2013) [17], soybean (Alidoust & Isoda, 2013) [2], rice (Alidoust & Isoda, 2014) [3], and root dry biomass of peanut (Liu *et al.* 2005) [19]. Pariona *et al.* (2017) [22] compared the iron sources like ferrihydrite and hematite and they found the highest root length and root mass in maize crop by the application of hematite treatment (4 g/L) that was increased 63 and 23 % respectively in relation to the control (p = 0.06). Correspondingly, 20 mg/L of γ Fe_2O_3 NPs significantly promoted root elongation by 11.5 % compared with control and suggested that cell elongation in the root system could lead to faster root growth (Li *et al.* 2016) [18]. It has been proved that a significant amount of Fe_2O_3 NPs has been directly taken up and translocated into different parts of the pumpkin plant when it suspended in a liquid medium or injected/sprayed on leaves as carbon-coated iron NPs (Li *et al.*, 2013) [17]. Moreover, root elongation and photosynthetic activity in soybean plants were significantly higher in γ Fe_2O_3 NPs via foliar spray as compared to soil route, which may be due to precipitation of Fe ions in soil (Alidoust & Isoda, 2013) [2] and adsorption of aggregated nanoparticles on root surface (Li *et al.* 2016) [18]. Among the various doses of iron fertilizers, 4g/lit was considered as an ideal concentration for increasing the shoot dry weight (40.7 g/plant) as well as root dry weight (3.8 g/plant) of cotton (Table 5).

Table 5: Mean comparison of yield parameters of cotton at post-harvest stage

Treatments		No. of symbol dial branches/plant	No. of total bolls/plant	No. of opened bolls/ plant	Seed cotton yield (g/pot)	Boll wt (g/boll)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Iron sources of fertilizers (F)								
Normal $FeSO_4$	F1	12.5	11.3	4.6	14.8	3.3	33.2	3.4
Normal FeO	F2	12.4	10.4	4.6	13.7	2.9	30.2	3.6
Nano Fe_2O_3	F3	13.7	12.0	4.7	13.7	3.0	36.6	4.5
Nano Fe_3O_4	F4	15.1	16.0	4.3	14.9	3.5	40.5	3.8
Control	F5	12.1	11.4	4.1	10.5	2.6	33.4	3.1
Doses of concentration (D)								
2 g/lit	D1	12.7	11.1	4.9	14.6	3.0	32.1	3.5
4 g/lit	D2	14.7	13.3	4.5	13.5	3.1	40.7	3.8
6 g/lit	D3	13.7	11.9	4.4	13.3	2.3	37.4	3.6
8 g/lit	D4	12.4	13.4	4.4	13.2	2.9	30.4	3.8
10 g/lit	D5	12.4	11.4	4.1	12.9	3.1	33.3	3.6

4. Conclusion

Iron oxide nanoparticles especially magnatite had significant effects on plant height, leaf area, seed cotton yield and boll weight, besides it had the positive impact on number of leaves, number of total bolls per plant and shoot dry weight of cotton. In contrary, haematite nanoparticles significantly influenced on root dry weight of cotton. This result showed the strong evidence on plant growth enhancement due to new type of iron nanofertilizer and these could replace the traditional fertilizers at lower concentration of 4 mg/lit. So, this magnetite nanoparticle might be considered as an ideal substitution for iron sulphate fertilizers on enhancing the seed cotton yield. Further research work on evaluating the effectiveness of iron oxide nanoparticles under field condition is required to recommend this new type of iron nanofertilizers for farmers. Besides, additional developments in agriculture nanotechnology could have multiplied the benefits to consumers, producers, farmers and environmental system.

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