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COMPARISON OF FOLIAR AND SOIL APPLICATIONS FOR CORRECTION OF IRON DEFICIENCY IN PEANUT (*ARACHIS HYPOGAEA* L.)

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Abstract

Pakistan has extensive iron (Fe) deficiency in its calcareous soils in Pothwar tract. The problem leads to Fe deficiency Chlorosis in peanut (*Arachis hypogea* L.) due to high pH and high bicarbonate levels. Foliar as well as soil applications of Fe-EDTA, $FeSO_4$ and sequestrene were used in pots for amelioration of Fe deficiency in already screened genotypes BARI-2000 (Fe deficiency tolerant) and BARD-699 (Fe deficiency sensitive). Pod number of BARI-2000 increased upto 36% by foliar treatments of Fe-EDTA as compared to control. Soil applications of $FeSO_4$ resulted 22% increase in pod number in BARI-2000. Pod number of BARD-699 was increased upto 62% and 52% as compared to control when Fe-EDTA and sequestrene were applied as foliar application. Strong correlation was found among photosynthetic rate and total Fe in BARI-2000. Similarly transpiration rate was positively correlated with total Fe and transpiration rate in BARI-2000. In BARD-699 strong correlation was found among photosynthetic rate and morpho-physiological parameters. Similarly transpiration rate was positively correlated with different parameters. Our results suggested that foliar applications were more effective in combating Fe deficiency in peanut.

Key words: Foliar applications, Soil applications, Peanut, Fe, $FeSO_4$

Introduction

Iron (Fe) deficiency is a key factor to reduce crop yield particularly growing in calcareous soils (Imtiaz *et al.,* 2010). Gris in 1843 established the essentiality of Fe in plants (Kumawat *et al*., 2006). Later a number of physiologists have explored the role of Fe in plant growth. Fe is one of the key plant nutrient, however, its physical and chemical properties make it difficult to uptake in plants from calcareous soil (Stephan, 2002). Numerous soil factors including pH (nearly 8.0), free $CaCO_3$ and HCO^-_3 affects the accessibility of Fe to plants (Kabata-Pendias, 2001). The problem is more pronounced in calcareous soils, where free $CaCO_3$ reacts with soil moisture and CO_2 to produce HCO^-_3 (Coulombe *et al*., 1984), which is the key factor in bringing Fe deficiency in crops growing in calcareous soils (Römheld & Marschner, 1986) .Other than inter veinal chlorosis, Fe deficiency decreases the activity of different enzymes including peroxidase and catalase, which contains porphyrin as prosthetic group (Hsu & Miller, 1968) ,these enzymes play important role in plant metabolism. Foliar spray in the form of Fe transporters to the soil may reduce the symptoms of Fe chlorosis to plants (Chen & Barak, 1982). Both methods are partly successful, as the addition of Fe to soil in the form of inorganic carrier is vulnerable to transform into inaccessible form in calcareous soils which limits Fe uptake by the roots of plants (Longnecker, 1988). Fe chlorosis is also induced by HCO^-_3 that impairs the mechanism for uptake of Fe (Coulombe *et al.,* 1984), therefore adding Fe to soil might not be able to diminish the effect of HCO^-_3. Spray of Fe salts alone is ineffective because, when applied to leaves in the form of sprays Fe get precipitated, because leaves lack acid producing mechanism as the roots have (Chen & Barak, 1982). Repeated soil applications of Fe-EDDHA or acidification can improve the uptake of Fe from calcareous soils (Rajaie & Tavakoly, 2018).

Poor translocation of Fe is another problem related to sprays in plants and frequent sprays may result in leaf injury (Singh *et al*., 2003). Citric acid is used to acidify the Fe solutions for foliar applications as low pH of acid spray solution increases uptake and transport of applied Fe in the plant (Tiffin, 1966). Combination of ferrous sulphate or other solutions with additional products may result in sustaining the added Fe source in accessible form for a long period of time (Kumawat *et al.,* 2006). Though, there are many disadvantages, still foliar sprays are commonly used to rectify the problem of Fe deficiency in plants (Roosta & Mohsenian, 2012; Schaffer *et al.,* 2011; Tagliavini *et al*., 2000). The foliar applications are used for treatment of abiotic stress in plants as they are cost effective (Noreen *et al.,* 2018). Experiments were performed to assess the effect of different soil and foliar applications for amelioration of Fe chlorosis of peanut in calcareous soils and also to compare commercially available chemicals for mitigation of Fe deficiency in the form of foliar sprays and soil treatments.

Materials and Methods

Five seeds of each of two selected genotypes of *Arachis hypogea* L. were germinated directly in pots in green house at National Agriculture Research Centre, Islamabad, Pakistan. Two genotypes were declared as Fe deficiency tolerant (BARI-2000) and Fe deficiency sensitive (BARD-699) based on previous hydroponics and pot experiments (Akhtar *et al.*, 2014; Akhtar *et al.*, 2013). Pots with 15 kg capacity were filled with soil and

sand in 1:1. NPK (20: 80: 20) were applied after seed sowing. The experiment was replicated thrice. In control, no foliar treatments were applied to plants seedlings, however for ameliorative purpose following treatments were used; soil application of 33.3mg per kg $FeSO_4$, soil application of 26.6.mg/kg Fe-EDTA, soil application of 67 mg per kg sequestrene {Na-FeEDDHA (ethylenediamine di (o-hydroxy-phenyl acetic acid) containing 6% Fe}, 0.5% foliar application of $FeSO_4$, 0.1mmol foliar treatment of Fe-EDTA and foliar treatment of 1%sequestrene. The treatments were applied at 45, 60 and 90 days after seed sowing.

Chlorophyll content was recorded at 45, 60 and 90 days after sowing (DAS) with chlorophyll meter SPAD-502 (Minolta, Japan) and was expressed as SPAD values. After sowing, Photosynthetic and Transpiration rates were recorded by using IRGA LCA4 at 45, 60 and 90. At 90 days after planting, active Fe concentration was measured (Gao & Shi, 2007). Before harvesting total Fe was measured by dry ash method (Rashid *et al*., 2001). The concentrations of active and total Fe were expressed in µg per g fresh and dry weight of plant material respectively. Pods number per plant were recorded. Pods weight and biomass was recorded after drying in sun for one day. Seed number and seed weight per plant was recorded. Percentage increase or decrease of different parameters was recorded under each treatment as compared to control as was shown on each bar. Data was subjected to Minitab 13 and Pearson's correlation coefficients were calculated.

Table 1. Physical and chemical properties of soil samples characteristics.

Depth	0-15cm	15-30cm
Clay %	17	18
Silt %	40	41
Sand %	44	42
Soil texture	Sandy loam	Sandy loam
ECe (dSm^{-1})	0.26	0.22
Soil pH	7.9	8.01
Bulk density (Mgm^{-3})	1.41	1.52
Soil moisture (g $100g^{-1}$)	8.92	9.30
Total N (µg/g)	153	155
Organic C (g $100g^{-1}$)	0.34	0.35
Available P (µg/g)	3.45	3.55
Extractable K (µg/g)	80	85
Fe (µg/g)	2.16	2.33
Zn (µg/g)	0.35	0.34
Mn (µg/g)	1.34	1.34
Cu (µg/g)	0.32	0.33
$CaCO_3$	5.6%	5.8%

Results

The soils of Pothwar region are calcareous in nature. The soil used in this experiment was with very low Fe content i.e., below critical level (Table 1).

Percentage (%) shows increase or decrease with reference to control. Maximum number of pods of BARI-2000 were recorded with foliar application of Fe-EDTA, where 36% increase in pod number was recorded when compared with control. Soil application of $FeSO_4$ resulted 22% increase in pod number as compared to control. Fe-EDTA applied to soil and $FeSO_4$ foliar application resulted decreased pod number of BARI-2000 when related to control. Foliar sprays of Fe-EDTA increased pod number of BARD-699 up to 62% as matched to control, while foliar application of sequestrene resulted in 52% increase in pod number. Foliar application as well as soil application resulted the increase in pod number of BARD-699 (Fig. 1).

Foliar application of Fe-EDTA showed 45% increase in pod weight, while 31% increase in pod weight of BARI-2000 was recorded with soil application of $FeSO_4$. Other treatments resulted increase in pod weight as paralleled to control. In case of BARD-699, all treatments resulted increase in pod weight more than 60% as matched to control. Foliar application of Fe-EDTA 81% increase in pod weight of BARD-699 was recorded while foliar application of $FeSO_4$ 78% increase in pod weight was found as matched to control. Foliar and soil application of sequestrene resulted 75% increase in pod weight in comparison to control (Fig. 2).

Foliar treatment of Fe-EDTA was more effective in increasing number of seeds per plant of BARI-2000 (55%) as well as BARD-699 (66%) as compared to control. Soil application of $FeSO_4$ also resulted 37% increase in number of seeds of BARI-2000, while soil application of sequestrene resulted 28% increase in number of nuts, when compared to control. Foliar application of sequestrene resulted 28% increase in number of nuts of BARI-2000 and foliar application of $FeSO_4$ resulted 34% increase in number of nuts as related to control. Other treatments also resulted more than 40% increase in number of seeds as compared to control (Fig. 3).

Seed weight of BARD-699 was increased up to 66% with foliar application of Fe-EDTA, while soil application of $FeSO_4$ resulted 39% increase in seed weight, when compared to control (Fig. 4). All treatments resulted in more than 70% increase in seed weight of BARD-699. Soil and foliar application of $FeSO_4$ resulted in 41 and 26% increase in seed weight of BARI-2000, while soil and foliar application of FeEDTA increased the nuts weight of BARI-2000 up to 18 and 54%. Soil and foliar application of sequestrene resulted in 39 and 19% increase in seed weight (Fig. 4). Its shows that foliar and soil application can increase seed weight and pods production. At three different days after sowing i.e 45, 60 and 90, SPAD values of BARD-699 were higher as compared to BARD-699 with all foliar as well as soil applications (Figs. 5,6 and 7). This showed BARD-699 responsiveness towards Fe deficiency.

Active Fe concentration of BARD-699 increased up to 44, 26%and 37% with foliar application of $FeSO_4$, sequestrene and Fe-EDTA as compared to control. BARI-2000 showed 28, 23 and 37% increase in active Fe concentration with same foliar applications. Soil application of $FeSO_4$ resulted 38% increase in active Fe concentration of BARI-2000 (Fig. 8), 70 and 63% with soil application of $FeSO_4$, sequestrene, Fe-EDTA and foliar application of $FeSO_4$, sequestrene and Fe-EDTA as compared to control. With same treatments, total Fe concentration of BARI-2000 improved up to 2, 18, 5, 21, 28 and 37% as compared to control (Fig. 9).

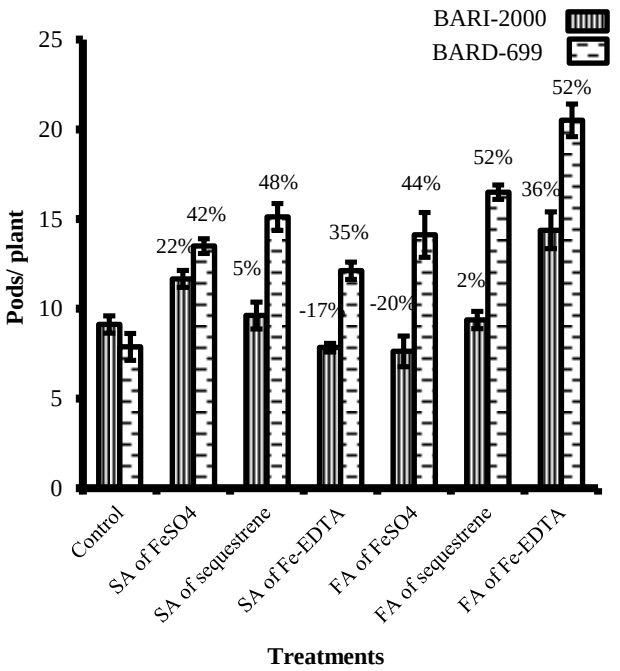

Fig. 1. Number of pods of peanut genotypes in soil culture under various soil and foliar treatments.

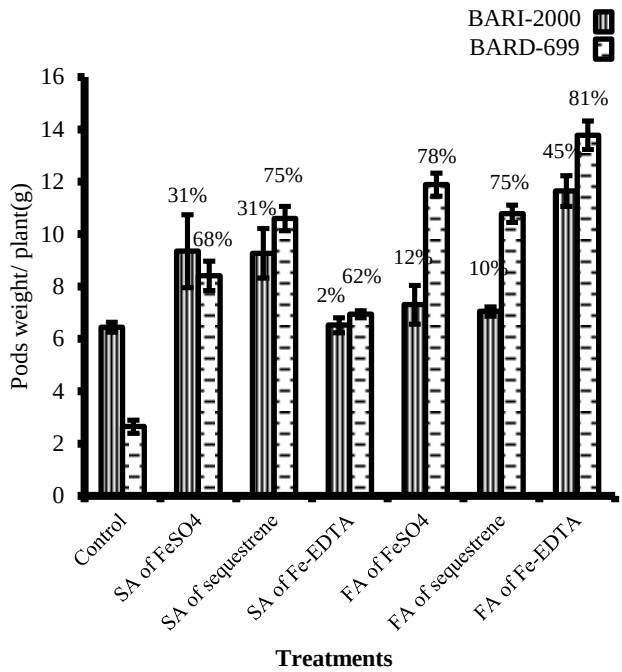

Fig. 2. Pods weight of peanut genotypes in soil culture under various soil and foliar treatments.

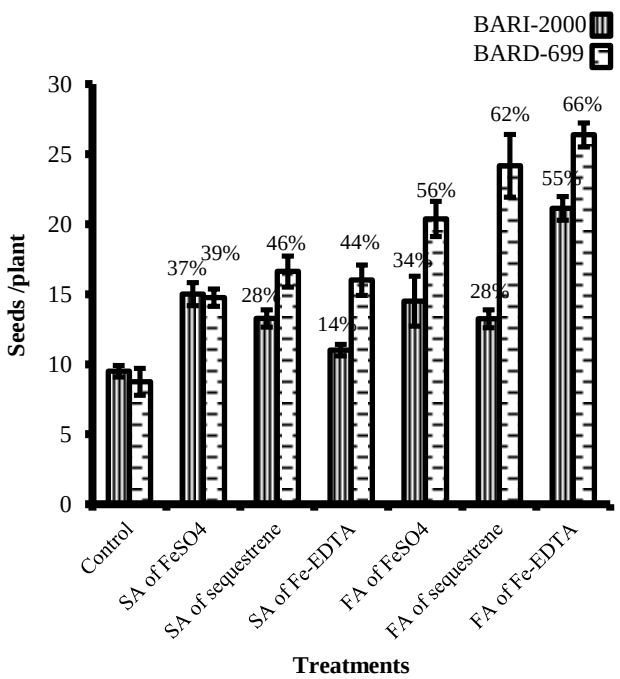

Fig. 3. Number of seeds of peanut genotypes in soil culture under various soil and foliar treatments.

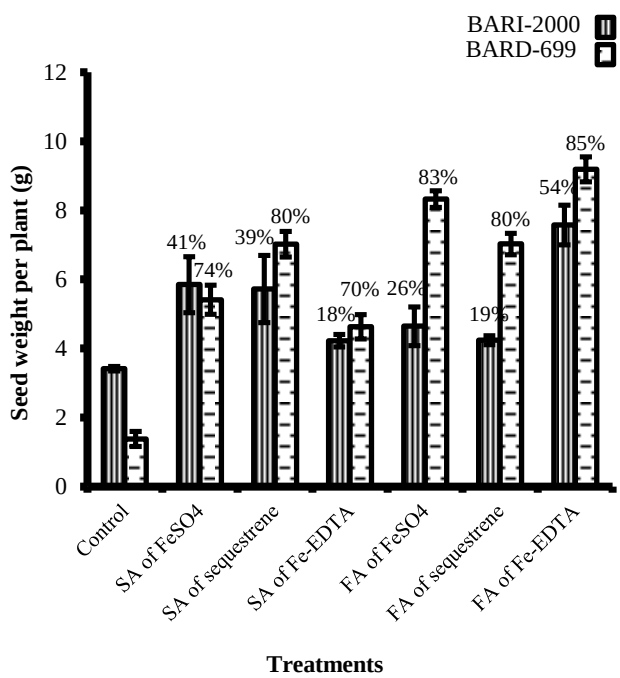

Fig. 4. Seed weight of peanut genotypes in soil culture under various soil and foliar treatments.

The results showed that foliar treatments were more effective in enhancing the yield and yield components as compared to soil application. Pearson's correlation coefficients showed significantly higher correlation between pod number and pod weight, number of seeds and seeds weight of BARI-2000 (Table 2). Similarly transpiration and photosynthetic rates were positively correlated with number of nuts and total Fe content. Photosynthetic rate was significantly correlated with transpiration rate in BARI-2000, however negative correlation was found between photosynthetic rate and transpiration rate with biomass (Table 2). Morpho-physiological parameters were significantly related to each other in BARD-699 (Table 3). Active and total Fe concentrations, Photosynthetic and transpiration rates were non-significantly related to biomass, however, it was significantly related with SPAD values, pod number, pod weight, number of seeds and seed weight (Table 3). Leaf dry weight was considerably higher under foliar application of $FeSO_4$ without surfactant. It means that surfactants could cause problem with Fe undertaking.

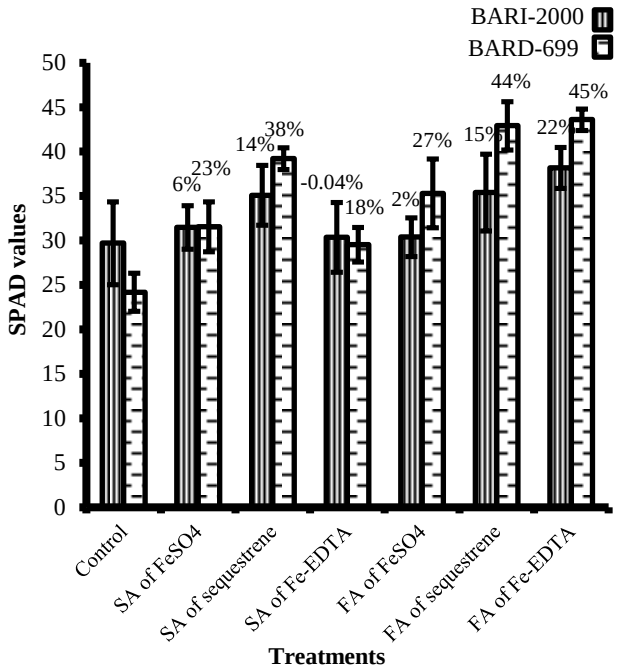

Fig. 5. SPAD values of peanut genotypes in soil culture under various soil and foliar treatments.

Discussion

Peanut is an important cash crop. The growing areas of peanut are calcareous in nature. Different foliar and soil applications can be helpful in reducing Fe deficiency chlorosis. Various researchers have studied the effect of foliar as well as soil applications to improve the Fe Chlorosis (Kumawat *et al.,* 2006; Rashid *et al.,* 1997). Our results indicated that BARD-699 was more responsive to the external application of Fe which showed its Fe deficiency behavior, which has already been proved by our previous experiments. Soil and foliar application of $FeSO_4$ increased the chlorophyll and active Fe as compared to control (Kumawat *et al*., 2006).

Soil applications of $FeSO_4$ and Fe-EDDHA proved to be more effective in recovering the deficiency symptoms of the young leaves of deciduous trees. $FeSO_4$ when added to manure was more effective in combating Fe deficiency. Our results are in line with the results of (Tagliavini *et al.,* 2000). The researchers showed that foliar application of $FeSO_4$ and citric acid was very effective in recovering the symptoms of iron deficiency as shown by SPAD values and Fe concentration. Soil treatment of Fe-EDDHA was very effective in re-greening of lychee plant as compared to other treatments including $FeSO_4$ and $FeSO_4$+surfactant shown by their chlorophyll index (Tagliavini *et al*., 2000). However, our results showed that foliar treatment of $FeSO_4$, Fe-EDTA and sequestrene were more active in making up of Fe deficiency chlorosis as compared to soil application. Among various treatments used, application of Fe-EDDHA to the soil increased Fe^{2+} values, however, soil as well as foliar treatments of $FeSO_4$ and surfactant increased the total and active Fe concentration. Soil Fe-EDDHA did not significantly increase the total Fe concentration (Tagliavini *et al*., 2000).

All plants in foliar application of $FeSO_4$ were greener (Schaffer *et al.,* 2011). Likewise, foliar application of $FeSO_4$ to pear trees improved the leaf chlorophyll content either due to the better activity of enzyme ferric chelate reductase after oxidation of foliarly applied Fe^{3+} to Fe^{2+} or the direct uptake of Fe^{2+} (Álvarez-Fernández *et al*., 2004). In soils mostly Fe is present in ferric form, however, the available form to plants is Fe^{2+} (Marschner *et al.,* 1986). Thus Fe^{3+} (ferric) must be reduced to Fe^{2+}(ferrous) in order to be taken up by plants (Brown, 2006; Chaney *et al.,* 1972). FRO and FRO homologues are responsible to reduce ferric to ferrous at the root surface (Ding *et al*., 2009; Waters *et al.,* 2002). Some of Fe is oxidized to ferric at the surface of root cells (Brown & Jolley, 1989). Outside the root cell, Fe^{3+} binds with root cell (Brown & Jolley, 1989). It is then transported in the form of ferric-citrate from root to the leaves and then it is reduced in the leaf apoplast to Fe^{2+}, that is vigorously transported into the symplast through the plasma membrane to be metabolized by the plants (Kosegarten *et al*., 2001). Like roots, ferric chelate reductase reduces the Fe in leaves (González-Vallejo *et al.,* 2000; Brüggemann *et al.,* 1993). In lychee leaves, foliar application of $FeSO_4$ either alone or in combination with acid and surfactant as well as with soil applied Fe-EDDHA leads to high leaf Fe^{2+} concentration. Total Fe concentration was higher than 110mg/kg DW in lychee plants with foliar application of $FeSO_4$ (Schaffer *et al*., 2011).

Previous findings supported our results that foliar applications are more effective in curing chlorosis. Often adequate amount of Fe is translocated to the leaves through roots, but the critical factor on Fe deficiency is the ability of leaves to metabolize Fe. This reduction is mainly dependent on pH and severely reduces at high pH (Mengel, 1994). Plants growing in calcareous soils have often high leaf apoplastic pH inhibiting the reduction of Fe^{3+} to Fe^{2+} (Mengel & Geurtzen, 2007; Mengel *et al*., 1994). It was also perceived that spraying chlorotic leaves of maize with indole acetic acid (IAA) or fusicoccin recovered the Fe deficiency symptoms (Mengel & Geurtzen, 2007). The researchers further related leaf apoplastic pH to Fe chlorosis as IAA and fusicoccin enhanced the secretion H^+ ions into the leaf apoplast by stimulating the plasma membrane proton pump. As a result pH was decreased. At low pH increased reduction of Fe^{3+} leads to higher uptake of Fe into the leaf symplast resulting in greater transport across the plasma membrane (Mengel, 1994).

The results suggested that foliar application was more active in combating Fe deficiency as compared to the soil applications of $FeSO_4$, Fe-EDTA and sequestrene. Plants usually respond to the foliar application of different acids resulting in re-greening of leave (Pestana *et al.,* 2001). However, foliar sprays of citric and sulphuric acid were not very active in re-greening of leaves in pear plants. The researchers suggested that number of foliar applications were related to the differences in species as well as physiological differences among genotypes and various morpho-physiological responses (Álvarez-Fernández *et al.,* 2004; Qian *et al*., 2018).

These results as well as the previous data suggested that external applications of different Fe sources reduced the pH of plant, as a result more mobilization of Fe in plants, finally led to re-greening process (Gao & Shi, 2007).

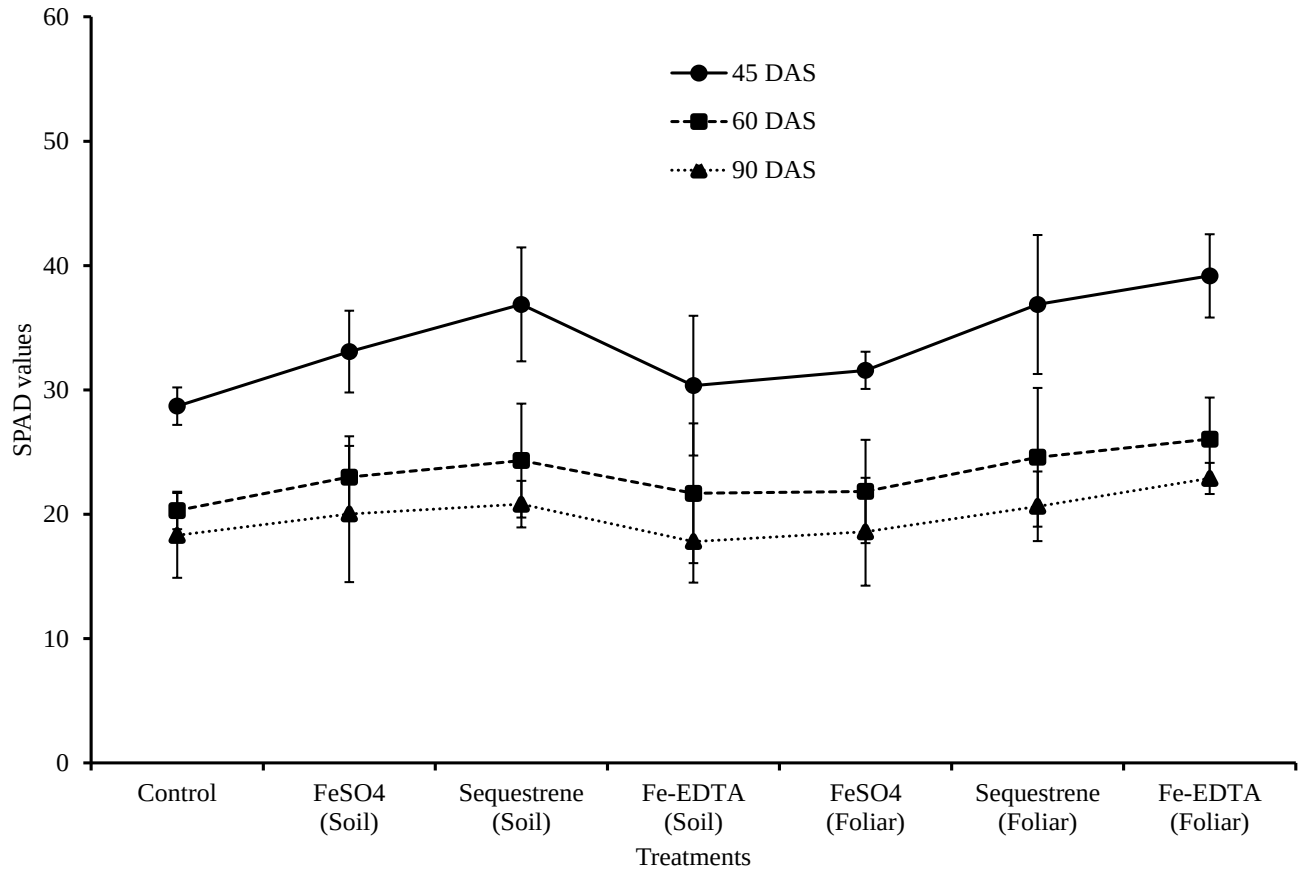

Fig. 6. SPAD values of BARI-2000 genotype in soil culture at different time intervals under various soil and foliar treatments.

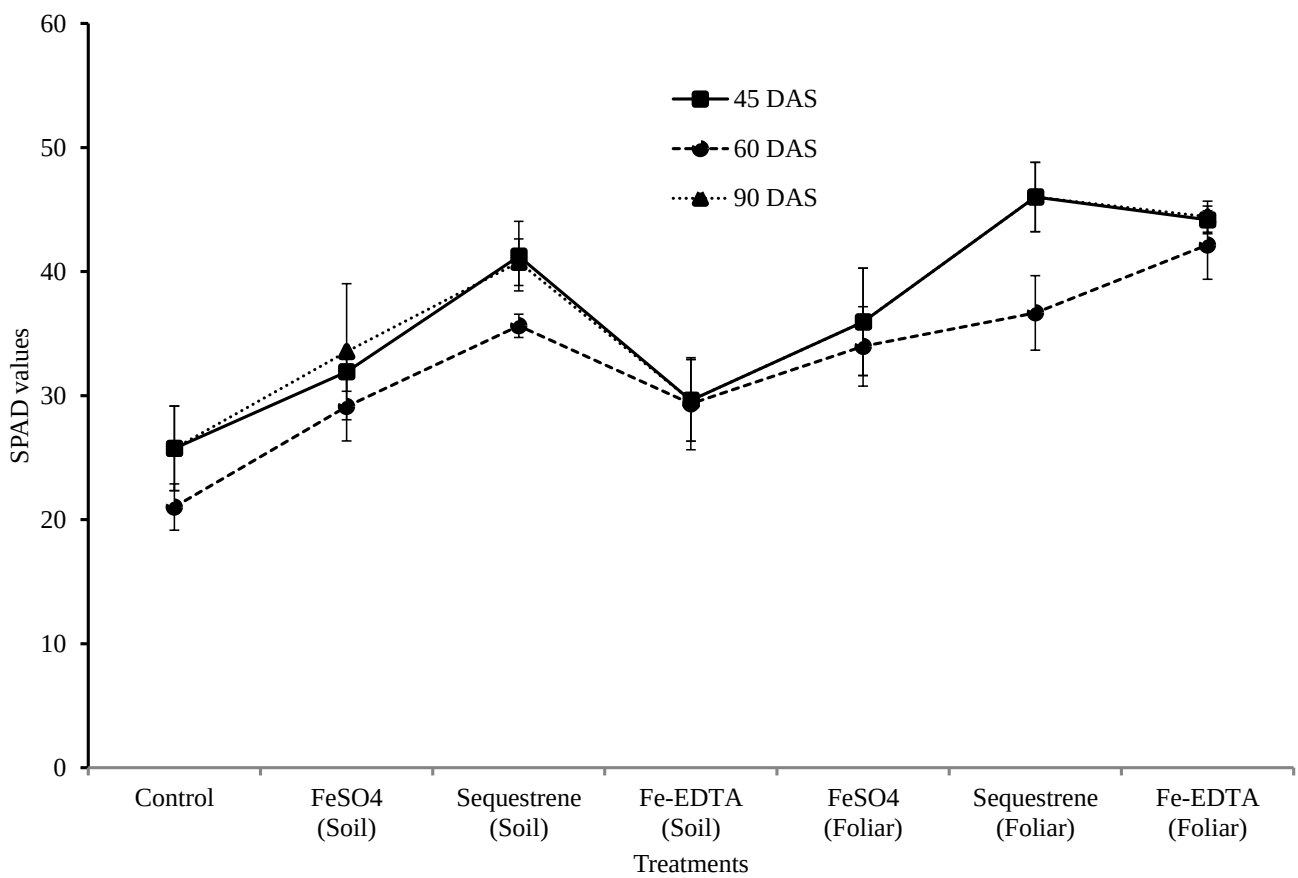

Fig. 7. SPAD values of BARD-699 genotype in soil culture at different time intervals under various soil and foliar treatments.

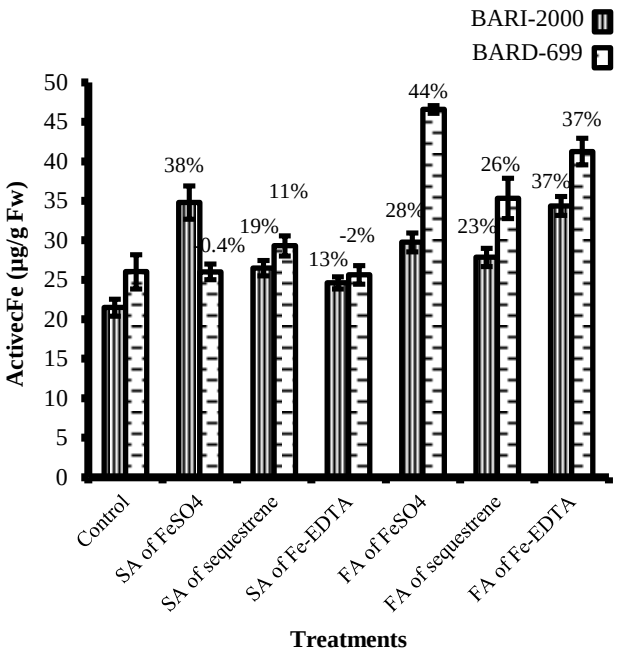

Fig. 8. Active Fe concentration of peanut genotypes in soil culture under various soil and foliar treatments.

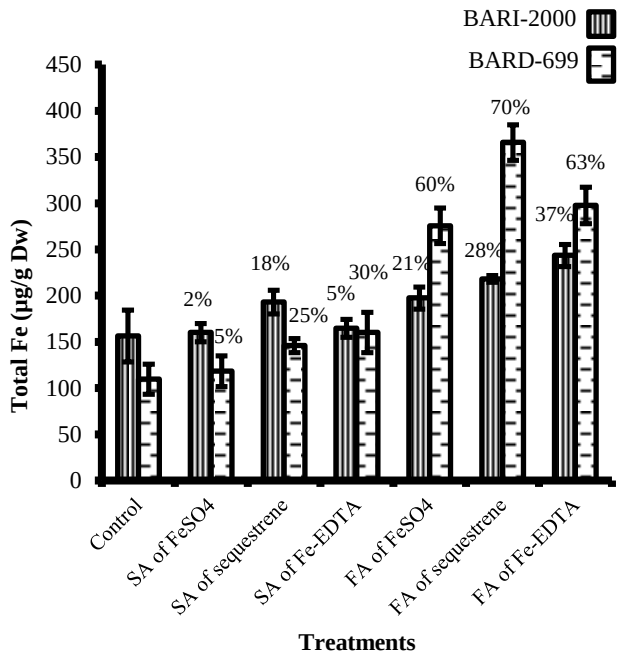

Fig. 9. Total Fe concentration of peanut genotypes in soil culture under various soil and foliar treatments.

Table 2. Pearson correlation coefficients of BARI-2000 along with probability of significance between different parameters recorded under control, soil and foliar applications.

	Biomass	Pod	No.of seeds	Pod wt	Seed_wt	shell_wt	Actv_Fe	T_Fe	SPAD	A	E
Biomass	1										
Pod	0.592**	1	0.770**								
No. of seed	0.335		1								
Psod_wt	0.477*	0.850**	0.831**	1							
Seeds_wt	0.374*	0.773**	0.850**	0.957**	1						
shell_wt	0.592**	0.852**	0.667**	0.893**	0.763**	1					
Actv_Fe	0.381*	0.660**	0.805**	0.780**	0.752**	0.636**	1				
T_Fe	0.207	0.471*	0.683**	0.464*	0.534**	0.326	0.435*	1			
SPAD	0.491**	0.485**	0.522**	0.526**	0.524**	0.454*	0.28	0.540**	1		
A	-0.038	0.292	0.638**	0.273	0.415*	0.056	0.392*	0.844**	0.419*	1	
E	-0.038	0.292	0.638**	0.273	0.415*	0.056	0.392*	0.844**	0.419*	1.000**	1

(*Data per plant Biomass; Number of pods; Number of seeds; Pods weight; Seed weight; Shells weight; Active iron; Total iron; SPAD: A; Photosynthetic rate, E; Transpiration rate)

Table 3. Pearson correlation coefficients of BARD-699 along with probability of significance between different parameters recorded under control, soil and foliar applications.

	Biomass	Pod	No.of seeds	Pod wt	Seed_wt	shell_wt	Actv_Fe	T_Fe	SPAD	A	E
Biomass	1										
Pod	0.709**	1	0.879**								
No. of seed	0.492**		1								
Psod_wt	0.685**	0.915**	0.889**	1							
Seeds_wt	0.654**	0.888**	0.877**	0.996**	1						
shell_wt	0.735**	0.949**	0.885**	0.975**	0.949**	1					
Actv_Fe	0.218	0.564**	0.729**	0.747**	0.771**	0.663**	1				
T_Fe	0.247	0.671**	0.875**	0.692**	0.686**	0.681**	0.746**	1			
SPAD	0.747**	0.892**	0.846**	0.857**	0.833**	0.883**	0.559**	0.727**	1		
A	0.345	0.665**	0.843**	0.685**	0.674**	0.687**	0.732**	0.969**	0.768**	1	
E	0.345	0.665**	0.843**	0.685**	0.674**	0.687**	0.732**	0.969**	0.768**	1.000**	1

(*Data per plant Biomass; Number of pods; Number of seeds; pods weight; nuts weight; Shells weight; Active iron; Total iron; SPAD: A; photosynthetic rate, E; transpirationte)

Conclusion

It is concluded from present study that foliar applications could be helpful to increase the yield of peanut in calcareous soils. Soil applications are less effective in combating Fe deficiency. $FeSO_4$ could be a good source to improve peanut yield if applied in the form of foliar sprays.

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