ROLE OF ORGANIC ACIDS IN AMELIORATION OF IRON CHLOROSIS IN GROUNDNUT (*Arachis hypogaea* L.) CROP GROWN IN A CALCAREOUS VERTISOL

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1. INTRODUCTION

Iron is the fourth most abundant element in the earth's crust after oxygen, silicon and aluminium where its concentration is 4.7 per cent by weight. The iron content expressed as per cent of Fe_2O_3 make up to one to six per cent of many soils which is comparable to seven per cent in earth crust and one to seven per cent in various rocks (Bear, 1964).

Many agricultural crops worldwide, especially in semi-arid climates suffer from iron deficiency. Among crop plants sensitive to iron deficiency are apple, avocado, barley, beans, citrus, grapes, oats, groundnut, soybean and numerous greenhouse flowers. Deficiency is usually recognized by chlorotic or yellow interveinal areas in new leaves and is typically found among sensitive crops grown in calcareous soils.

Iron is an essential micronutrient absorbed by plant in the form of Fe^{2+} ion. In plant, iron plays a role in the synthesis of chlorophyll, carbohydrate production, cell respiration, chemical reduction of nitrate and sulphate and in nitrogen assimilation. Iron is also found in haeme compounds such as various cytochromes, peroxidase and catalase and in phytoferritin and ferrodoxin. These compounds may have reduced activities under condition of iron deficiency (Agarwala and Sharma, 1961).

Calcareous soils cover over 30 per cent of the earth's land surface. Nutrient management in calcareous soils differs from that of non-calcareous soils because of the effect of soil pH on soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients. Iron deficiency is common in soils that have a high $CaCO_3$ due to reduced solubility of iron at alkaline pH values.

Calcareous soils may contain high levels of total Fe, but in forms unavailable to plants. However, owing to the nature and causes of Fe chlorosis, in chlorotic plants Fe^{3+} concentration can be higher than those in normal plants. Thus, this disorder in calcareous soils is not always attributable to Fe-deficiency, this condition is known as lime induced iron chlorosis.

Iron is considerably less soluble than Zn or Mn in soils with a pH value of 8, thus inorganic Fe contributes relatively little to the Fe nutrition of plants in calcareous soils. Most of the soluble Fe in the soil is complexed by natural organic compounds. The primary factor associated with iron chlorosis under calcareous condition appears to be the effect of the bicarbonate ion (HCO_3^-) in reducing Fe uptake and translocation to the leaves.

Iron deficiency seems to be one fourth as extensive as that of Zn, amongst the micronutrients. It is an important problem in Haryana and to a lesser extent in Tamil Nadu and Punjab. In Karnataka state, the results reported by the research institutions and the state Department of Agriculture, indicate that 39 per cent of soil samples collected from various parts of the state found to be deficient in iron.

Groundnut is one of the most important oilseed and food crops. India occupies the first position in the area and second position with regards to production of groundnut in the world. In India, groundnut is grown over an area of 7.0 million ha with the production of 8.8 million tones and productivity range of 770-1200 kg ha^{-1}. Andhra Pradesh, Gujarat, Karnataka and Maharashtra are the four states where 70 per cent of the area and 75 per cent of production of groundnut are concentrated in India.

Iron deficiency in extreme cases may lead to complete crop failure. For instance, Papastylianou (1990) in Cyprus observed complete groundnut failure grown in calcareous soils ($CaCO_3$ 46%) and recorded pod yield of 30 kg ha^{-1} and suggested that production of the untreated plants in such condition is trivial.

Two principle methods of treating iron deficiencies are accepted at present. Spraying foliage with inorganic salts has been found to be beneficial, but often gives spotty results due to limited penetration of iron into leaves. Also, repeated treatments are required during course of canopy development. Soil treatments with synthetic chelates have been found to be an almost unqualified success because of their high costs. Therefore, research continues for the lowest, effective remedy to iron deficiency.

Hence, an attempt will be made to study the effect of low cost remedial methods like organic acids as foliar spray. GPBD-4 an iron efficient variety and TMV-2 an iron inefficient variety grown in calcareous vertisol were used as test crops with the following objectives.

1. To understand the mechanisms of iron utilization in Fe deficient plants as influenced by foliar spray of organic acids
2. To study the amelioration effect of organic acids on Fe content and yield of groundnut crop.

2. REVIEW OF LITERATURE

Calcareous soils are widely spread in different regions and comprise over one-third of world's land surface area. Calcareous soils have free calcium carbonate in the profile. Nutrient management in calcareous soils differs from that in non-calcareous soils because of the effect of soil pH on soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients. Iron deficiency is common in soils that have a high $CaCO_3$ due to reduced solubility of iron at alkaline pH values. The chlorosis due to iron deficiency of groundnut crop grown in calcareous soil is a common problem causing slow development of plant and yield reduction. Literature pertaining to different aspects of iron nutrition in groundnut crop in calcareous Vertisol is reviewed and presented below under different heads.

2.1 Origin and characteristics of calcareous soil

2.2 Iron in plant nutrition

2.3 Iron availability in soil

2.4 Correction of iron deficiency

2.1 Origin and characteristics of calcareous soil

A calcareous soil is a soil that has free $CaCO_3$ in the profile that contains enough $CaCO_3$ so that it effervesces when treated with hydrochloric acid. When free carbonates are present, addition of acid results in bubbling due to the evolving of CO_2 gas (Loeppert and Suarez, 1996).

Hagin and Tucker (1982) define calcareous soil as a soil that its extractable Ca and Mg levels exceed the cation exchange capacity. The carbonates due to their relatively high solubility, reactivity and alkaline character buffer the pH of most calcareous soils within the range of 7.5 to 8.5. These soils generally have 100 per cent base saturation and the exchange complex is dominated by calcium.

Jackson and Erie (1973) reported that in some soils the $CaCO_3$ deposits are concentrated into layers that may be very hard and impermeable to water. These *caliche* layers are formed by rainfall leaching the salts to a particular depth in the soil at which water content is so low that carbonates precipitate. Soils can also become calcareous through long period of irrigation with water containing dissolved $CaCO_3$ (Hagin and Tucker, 1982).

Brady and Weil (1999) reported that calcareous soils occur naturally in arid and semi-arid regions because of relatively little leaching. They also occur in humid and semi-arid zones if their parent material is rich in $CaCO_3$, such as limestone, shells or calcareous glacial tills and the parent material is relatively young consequent to less weathering.

2.2 Iron in plant nutrition

Iron is one of the essential micronutrients required by the plants and its essentiality as micronutrient for plants was established in 1843 by E. Gris. Iron plays an important role in the synthesis of chlorophyll, carbohydrate production, cell respiration, chemical reduction of nitrate and sulphate and in N assimilation. With this in view, the available literature pertaining to iron in plant nutrition has been reviewed under the following sub-headings.

2.2.1 Metabolic functions of iron

Iron deficiency in plants typically causes chlorosis of leaf tissue because of inadequate chlorophyll synthesis. In a healthy plant, 60 per cent of all leaf iron is concentrated in chloroplast (Whatley *et al.*, 1951). The exact role of iron in chlorophyll synthesis is not certain but there is evidence for the involvement of ferrous iron in the condensation of succinic acid and glycine to form γ-aminolevulinic acid, which is condensed to form pyrrole groups, which in turn are condensed to form protoporphyrin IX. Magnesium is then incorporated into the molecule to form chlorophyll, possibly with the catalytic action of iron.

Iron is also found in heme compounds, such as the various cytochromes, peroxidases and catalases and in phytoferritins and ferredoxins. These compounds may have reduced activities under conditions of iron deficiency (Agarwala and Sharma, 1961).

2.2.2 Mechanisms of iron uptake

Relatively little is known about the mechanism of iron uptake by plant in soil.

Oliver and Barber (1966) reported that out of total Fe uptake by soybean, mass flow accounted for 3 to 9 per cent another 23 to 56 per cent of iron uptake was contributed by root interception.

O'Connor *et al.* (1971) pointed out that if mass flow alone were to be responsible for iron uptake of a plant transpiring at a rate of 500 g water per g dry matter and accumulating 100 µg Fe per g dry matter, the soil solutions would have to contain 0.2 ppm Fe.

Chaney *et al.* (1972) reported that iron must be reduced to Fe^{2+} at the root surface in order to be absorbed. A number of studies using nutrient culture demonstrate a drop in the redox potential of the solution. Calculation based on equilibrium with ferrihydrite at pH 7.0 show that redox potential (Eh) must drop to –10 mV, or Pe must be –0.2, in order to mobilize 0.2 ppm Fe as Fe^{2+}. Interestingly, poor soil aeration in neutral and calcareous soils, which is favourable to the drop in redox, has nonetheless often been linked to the appearance of iron chlorsis (Wallace and Hunt, 1960).

Olsen and Brown (1980) reported that mobilization of iron at the root-iron oxide interface seems to be achieved by either pH reduction or a drop in redox potential, or both depending upon plant species and variety.

Basavaraj and Uppar (2008) conducted a pot culture experiment (imposing three moisture and five $CaCO_3$ levels) at MARS, Dharwad to know the uptake of iron and calcium by groundnut crop in calcareous soils. After harvest of the crop, both forms of iron oxides in soil was found to decrease significantly with increased level of $CaCO_3$ and moisture level. With increasing level of $CaCO_3$, concentration of water soluble Fe decreased. Decrease in concentration of exchangeable Ca and DTPA-Fe was observed with increasing level of $CaCO_3$.

2.2.3 Causes of iron deficiency

Yaalon (1957) reported that 10 per cent active lime was the critical level in sensitive crops.

Miller (1960) reported that the precise manner in which HCO_3^- is harmful to iron nutrition is uncertain, but clearly the pH buffering effect of bicarbonate would be deleterious if a significant portion of plant iron is absorbed from iron oxides necessitating pH reduction at points of root contact.

Wallace and Hunt (1960) and Miller (1960) reported indicative of the respective role of soluble calcium, HCO_3^-, soil CO_2 and phosphorus in the induction of chlorosis.

The soil solution composition may influence plant nutrition in such a way as to induce chlorosis. Plants as a rule absorb more anions than cations to maintain electrical neutrality, the root must exude OH^- in amounts equivalent to the excess of anions over cations. In the presence of water and CO_2, OH^- forms bicarbonate (Cunningham, 1964).

2.2.4 Indicators of iron deficiency

North and Wallace (1952) found that high nitrate absorption caused chlorosis in avocado and therefore suggested the use of Fe/N as a measure of iron deficiency. The degree of chlorosis was related to the ratio of P to Fe for mustard seedlings grown in nutrient solutions (De Kock, 1955).

Agarwala and Sharma (1961) reported that most straight forward approach to quantifying iron chlorosis is determination of chlorophyll content of leaf tissue. The similar results were found for citrus that leaf chlorophyll is strongly related to the iron supply in the nutrient solution.

Katyal and Sharma (1980) reported that chlorotic leaves may contain as much or more iron than healthy leaves and that total iron may show no relationship to the chlorotic or healthy appearance of the plant. This is supported by De Kock (1955), Agarwala and Sharma (1961). In order to overcome this inconsistency, a new technique of fraction of plant iron analysis (Fe^{2+}) which involved in the synthesis of chlorophyll has been developed.

2.3 Iron availability in soil

Swaine (1955) and Hodgson *et al.* (1972) have given an authoritative and extensive account of the occurrence of micronutrients in soils. Kanwar and Randhawa (1974) have reviewed the research on micronutrients in soils and plants in India. The main source of micronutrients in soil is the parent material. From the nature of the parent material of the soil, it is possible to form an idea of the possible deficiency or excess of micronutrients in it.

Calcareous soils are characterized by high carbonates content, high pH and high bicarbonate in the soil. So, the pH of the plant system grown on these soil will increases resulting in lesser reduction of Fe^{3+} by Fe-reductase located in plasma membrane, which is pH dependent (Mengel, 1994).

Mengel and Kirkby (1996) reported that iron chlorosis may result from an absolute Fe deficiency in the soil, such cases may occur on degraded sandy soils, but are not frequent. Iron chlorosis on calcareous is not caused by absolute Fe deficiency. Calcareous soils may contain high levels of total Fe, but in forms unavailable to plants. Visible Fe-deficiency or Fe chlorosis is common in many crops. However, owing to the nature and causes of Fe chlorosis, leaf Fe concentration is not necessarily related to degree of chlorosis. In chlorotic plants Fe concentration can be higher or lower than those in normal plants. Thus, this disorder on calcareous soils is not always attributable to Fe deficiency but is rather a physiological disorder.

Tandon (1998) reported the lime induced iron chlorosis is a common problem when crop are grown on calcareous soils. Some of the crops which are sensitive to iron deficiencies are citrus, fieldbean, grapes, corn, bushbean, vegetables, ornamentals, straw berries and avocado.

It is known that none of them directly induced Fe chlorosis. The most important factor which brings about lime induced chlorosis is HCO_3^-. This ion affects iron uptake and translocation in the plant (Rutland, 1971).

Hence, factors limiting iron supply to crop plant are discussed under following headings.

2.3.1 Soil factors

2.3.2 Plant factors

2.3.3 Nutritional factors

2.3.4 Microbial factors

2.3.5 Environmental factors

2.3.1 Soil factors

Nutrient management in calcareous soils differs from that of non-calcareous soils because of the effect of soil pH on soil nutrient availability and chemical reactions that affect the loss or fixation of almost all nutrients.

2.3.1.1 Effect of $CaCO_3$ on iron availability

Thorne and Wann (1950) reported that high pH and excess quantities of lime make soil iron less available to plants. The most wide spread incidence of iron deficiency in plants occurs in alkaline calcareous soils.

Agarwala and Malhotra (1963) have noticed large number of plant species in Lucknow and its neighbourhood showing chlorosis, particularly in the young foliage. Higher incidence of chlorosis was noticed in locations where $CaCO_3$ content varied from 2.5 to 11.4 per cent and chlorosis was absent in locations where $CaCO_3$ content varied from 0.13 to 0.16 per cent. The chlorotic plants had as much and even more Fe than the comparable normal leaves. It showed that an insufficient supply of iron as well as the inactivation of iron after it was absorbed accounted for chlorotic condition.

Koraddi *et al.* (1969) conducted that one of the factors responsible for iron chlorosis of hybrid jowar in soils of Bijapur was due to very high $CaCO_3$ percentage with increase in $CaCO_3$ the available iron content was found to decrease in the medium black, mixed red and black soils.

Seatz and Peterson (1964) showed reaction between iron and carbonate ions may be a factor in reducing iron availability to plants.

$Fe^{2+} + CaCO_3$	$\rightleftharpoons$	$FeCO_3 + Ca^{2+}$
Ferrous iron		Siderite
$4FeCO_3 + 2H_2O + O_2$	$\longrightarrow$	$4Fe(OH)CO_3$
Ferrous carbonate		Ferric carbonate
$Fe(OH)CO_3 + 2H_2O$	$\longrightarrow$	$Fe(OH)_3 + H_2CO_3$
		Ferric hydroxide

Singh and Dahiya (1975) in Hissar, Haryana conducted experiment to find out the effect of added $CaCO_3$ and iron on the translocation of iron in a light textured soil in laboratory. Exchangeable, available and reducible iron decreased with increase in added $CaCO_3$. Iron increases in all forms irrespective of addition of $CaCO_3$.

Singh and Patiram (1975) noticed a significant negative correlation between available iron and $CaCO_3$. It was probably due to precipitation and absorption of iron in the presence of $CaCO_3$.

Lindsay and Schwab (1982) concluded that Fe^{2+} activity in a highly reduced soil was controlled by $FeCO_3$ (siderite) at low redox levels (Pe + pH <8.5). While Fe^{2+} activity was controlled by $Fe_3(OH)_8$ (ferrosic hydroxide) above this redox level.

Joshi *et al.* (1983) observed negative dominant influence of $CaCO_3$ on DTPA extractable iron in coarse and medium textured soils.

Dhange *et al.* (1985) noticed a significant negative correlation (r = -0.617**) between $CaCO_3$ and DTPA-extractable iron in soils of Rahuri. Sakal *et al.* (1985) also observed similar results in calcareous soils of Bihar. Singh *et al.* (1988b) found significant negative correlation (r = -0.34) between available Fe and $CaCO_3$ content of soils of Haryana.

Papastylianou (1989) reported various degree of chlorosis based on survey conducted in peanut (*Arachis hypogaea* L.) fields. In the area concerned, plant appearance was classified according to a 'chlorotic index' and corresponding soil samples were taken and analysed for free $CaCO_3$, pH, NO_3-N, DTPA extractable Fe and active lime. Results from regression analysis shows that $CaCO_3$, active lime and NO_3-N were positively correlated, while DTPA extractable Fe was negatively correlated with degree of chlorosis. Further, author reported that plants appear to be a chlorotic when DTPA-Fe was below 2.5 ppm.

Patiram and Rai (1989) reported that liming the soils decreased significantly the available Fe in the soil which was probably due to precipitation of Fe as carbonates, oxides or hydroxides resulting from an increase in pH after liming.

Suthar and Patel (1992) in a pot experiment using a calcareous vertic ustochrept soil studied the effect of lime and soil water on groundnut. Lime application decreased pod yield, available Fe in soil and Fe, Mn and K contents of plant but increased P content.

Based on results of long-term experiment on maize-wheat sequence was initiated in 1972-73 on a Typic Hapludalf at Palampur, Himachal Pradesh. Deepak Kher (1993) reported that application of lime decreased the available Fe and Mn significantly by 40 and 11 per cent, respectively.

2.3.1.2 Iron availability as influenced by $CaCO_3$ along with moisture

A pot culture experiment was conducted in calcareous vertic Ustochrept at Junagarh, Gujarat by Suthar and Patel (1992). They studied the effect of lime (0, 10 and 20%) and soil water (saturation, 20 and 80% of soil water) on groundnut. Lime application decreased pod yield and the soil available iron and increased the plant Fe, Mn and Zn contents.

Sarkar (2000) conducted a pot culture experiment imposing three moisture and five $CaCO_3$ levels in Dharwad to know the concentration and uptake of iron as influenced by above treatments. At the harvest of the crop concentration and uptake of iron increased but yield decreased with increase in $CaCO_3$ and moisture level.

Basavaraj and Uppar (2008) conducted a pot culture experiment to know the soil factors influencing iron availability and translocation of iron in plants and groundnut (JL-24) was used as a test crop. The soils were selected from four places namely Chilakwad and Yamanur (Navalgund taluk), Kalasapur and Nagavi (Gadag taluk) which represents four level of $CaCO_3$ (8.8, 12.8, 18.0 &23.0 %). Plants grown in pots were analysed for ferrous (Fe^{2+}), ferric (Fe^{3+}) and total soluble iron in younger leaves, older leaves and stem. Ferrous iron concentration decreased with increase in $CaCO_3$ level even with the increase in ferrous iron concentration from 60 to 70 days after sowing (DAS), the plants are considered as chlorotic. Haulm yield was not affected significantly from 10.29 to 8.27 mg/pot with increase in $CaCO_3$ levels.

2.3.1.3 Iron availability as influenced by bicarbonate ion

Iron is considerably less soluble than Zn or Mn in soil pH of 8, thus inorganic Fe contributes relatively little to the Fe nutrition of plants in calcareous soils. The primary factor associated with Fe chlorosis under calcareous conditions appears to be the effect of the bicarbonate iron (HCO_3^-) in reducing Fe uptake and translocation to the leaves. Besides the inability of the plant to take up iron from the soil, iron deficiency may also be due to inactivation of the iron within the plant.

Porter and Thorne (1955) stated that excess irrigation worsened Fe chlorosis because of high HCO_3^- in the soil solutions rather than low O_2 levels. Agarwala and Malhotra (1963) were of opinion that bicarbonate was the major causative factor for iron chlorosis in calcareous soil.

Boxma (1972) conducted pot experiment and field trials to determine the direct cause of lime-induced chlorosis in Netherlands. He concluded that high HCO_3^- content in the soil was the main cause and there was a significant negative correlation between lime-induced chlorosis and the HCO_3^- content of the soil.

Mengel *et al.* (1984) conducted a pot culture experiment using grape wine crop on a calcareous and non-calcareous soil with low and high water saturation. Soil saturation incraese pH and HCO_3^- ion in both soils, but this level was much higher in calcareous soil. Soil and plants were also analysed for Fe and P after 3 weeks of growing period. They confirmed that HCO_3^- was the primary cause for iron chlorosis but not phosphorus.

Coulombe *et al.* (1984) also studied on bicarbonate induced iron chlorosis. Four soybean cultivars differing in susceptibility to Fe-chlorosis in wet calcareous soils were grown in nutrient solutions to characterize the effects of phosphorus and HCO_3^- in inducing chlorosis. $NaHCO_3^-$ (0 or 10 μM), P (10 or 400 μM) as NaH_2PO_4 and NH_4^+ (0 or 300 μM) as $(NH_4)_2SO_4$ were factorial treatments. In a nutrient solutions (5 μM Fe + 10 μM EDDHA). With no HCO_3^- added, 'T203' (extremely chlorosis susceptible) was green at 10 μM P, but severely chlorotic at 400 μM P; 'Wayne' (chlorosis susceptible) was green at both P levels. Addition of HCO_3^- caused chlorosis in T203 and Wayne at 10 to 400 μM P. Chlorosis resistant '4P9' and 'Hawakye' remained green with added P or HCO_3^-. Low Fe concentrations in younger leaves corresponds with added P or HCO_3^- was observed. Low concentration in young leaves did not change with HCO_3^- at 10 μM P, but decreased with HCO_3^- at 400 μM P. In the absence of HCO_3^- 'Wayne' was not chlorotic at either concentration of solution P, although this cultivar is known to be highly susceptible to chlorosis in the field. HCO_3^- induced chlorosis was not the result of increased solubility on plant uptake of P. Thus, HCO_3^- was a direct factor in causing soybean chlorosis.

Rao *et al.* (1993) reported that the iron chlorosis common in groundnut grown in calcareous soils. They observed chlorosis particularly in over irrigated fields. They studied the correlation between several factors induces iron chlorosis and found strong significant positive correlation between soil moisture and HCO_3^- (r = 0.96) and strong negative significant correlation between soil HCO_3^- and Fe^{2+} in plant (r = -0.94).

Mengel (1994) conducted a lab experiment and reported that iron chlorosis can commonly occur in plants grown on calcareous soils. Such soils may contain high HCO_3^- concentration in their soil solution. They are characterized by a high soil pH and they rather tend to accumulate nitrate than ammonium because of high pH. Hence, in these soils plant roots may be exposed to high nitrate and high HCO_3^- concentrations. Both anion species are involved in the induction of iron chlorosis. Iron transport across the plasma membrane is initiated by Fe^{3+} located Fe^{3+} reductase. So, he confirmed that there is a negative and significant correlation between the leaf apoplast pH and the degree of iron chlorosis measured as leaf chlorophyll concentration. Depressing leaf apoplast pH by simply spraying chlorotic leaves with an acid led to a regreening of the leaves.

2.3.1.4 Iron availability as influenced by soil pH

Dharmiji *et al.* (1956) pointed that there is abundant evidence that there exists a definite relationship between the Fe^{2+} content which is considered as the available form of Fe and soil pH and they found existence of negative correlation between the available iron and soil pH.

Singh (1964) reported that Fe^{2+} content of the soil increased with decreasing pH. Agarwala *et al.* (1964) observed a negative relationship (r = -0.69) between ESP and exchangeable Fe content of an Usar soil from Uttar Pradesh.

As the pH increases to 6.5 and above soluble ferrous iron (Fe^{2+}) tends to become oxidized to ferric iron (Fe^{3+}), which being insoluble under normal and alkaline conditions leads to iron deficiency. The soluble iron level reaches a minimum in pH range between 6.5 and 8.0 (Lindsay, 1972).

Lindsay (1972) reported that to enable transport of sufficient iron to plant, the total solubility must be at least 10^{-6} M. At normal pH solubility of iron is far below from plant requirement. Each unit increase in pH decraeses the solubility of ferric iron as much as 1000 times and ferrous iron by 100 times (Lindsay and Schwab, 1982).

Verma and Tripathi (1982) studied the plant micronutrients relationship with soil pH. They found that plant iron had a negative and significant coefficient of regression with soil iron in soils of pH 8.5, but positive in soils of pH 6.5. Kannan (1984) was of the opinion that the soils in India where iron chlorosis is observed have high calcareousness with pH>8.

Clemens *et al.* (1987) investigated soil conditions leading to chlorosis symptoms on plant for a number of arid and semi-arid soil types using groundnut as indicator plant. The occurrence of chlorosis could be described as a functions of $CaCO_3$, Fe supply and pH.

Rajkumar *et al.* (1990) studied the depth wise distribution of four available micronutrients in soil series of Bundelkhanda region of Madhya Pradesh and reported that available Fe and Mn increased with depth and pH was found to be dominant factor controlling the availability of Fe and Mn in these soils.

Prasad and Sakal (1991) reported that iron was extracted from calcareous soils using DTPA-$CaCl_2$ (pH 7.3), DTPA-$CaCl_2$ (pH 8.0), EDTA $(NH_4)_2CO_3$ (pH 8.6), DTPA $(NH_4)_2CO_3$ (pH 7.6) and EDTA NH_4OAc (pH 7.0). Iron availability was negatively correlated with pH and $CaCO_3$ and positively correlated with organic carbon.

Khorsandi (1994) studied the effect of sulphuric acid on the availability of Fe and P in two calcareous soils and concluded that the change in soil pH was the key to the increased nutrient availability and subsequent crop yield. Thakur *et al.* (1995) noticed that the reduced availability of iron with increase in pH.

To find out the cause for iron deficiency in the Vertisol (Typic Chromousterts) in Maharashtra. Yelvikar *et al.* (1996) analyzed several soil samples and found significant influence of pH, $CaCO_3$ organic carbon and clay to different fractions of iron.

Rao and Rao (1994) in a hydroponic culture studied the effect of iron stress on growth and inorganic composition of groundnut cv. TMV-2, was supplied with 5 ppm Fe at pH of 5.5, 6.5, 7.5 and 8.5. The seedlings grown at pH 7.5 and 8.5 produced severe Fe chlorotic symptoms.

Iron deficiency decreased root growth more than shoot growth in groundnut and it caused 3 to 4 fold reduction in photosynthetic rate and chlorophyll content in the legumes. They also reported that physiologically active Fe^{2+} decreased markedly by increased pH but not total Fe.

2.3.1.5 Iron availability as influenced by organic matter and clay content

Saha *et al.* (1982) obtained significant positive correlation with available iron and organic carbon and clay content in soils of Assam and West Bengal. Similar results were obtained by Sakal *et al.* (1985), Dhange *et al.* (1985) and Yelvikar *et al.* (1996).

Loeppert and Hallmark (1985) observed positive correlation of clay and organic matter contents to plant available Fe. Katyal and Sharma (1991) observed positive relationship of DTPA-Fe with organic matter in soil in different zones of India.

Loeppert (1986) found that plant iron status in the soil by exudation of proton and chelate and/or development of an increased capacity for reduction of Fe. These factors will act to mobilize labile Fe from solid phase soil components especially the poorly crystalline iron oxides. Therefore, the crystellinity particle sizes and reactive surface area of the soil iron oxides influence the availability of Fe to plants in calcareous soils.

Raut *et al.* (1998) conducted a survey work in groundnut growing areas of Jayakwadi Command Area of Maharashtra. They analyzed one hundred surface soil samples for iron status different in soil characteristics and correlation coefficients between total Fe and available iron. There was significant negative correlation with pH, $CaCO_3$ and sand. Whereas, organic carbon, clay and available N had significant positive correlation.

2.3.1.6 Iron availability as influenced by soil moisture

Under reduced conditions iron will be present in ferrous form and is available to plants. Thorne and Wann (1950) estimated that 50 per cent of the chlorosis in Utah could be prevented by controlling the soil moisture conditions.

Porter and Thorne (1955) stated that manure and/or excess irrigation worsened Fe chlorosis because of high HCO_3^- in the soil solution rather than low O_2 levels.

Grass *et al.* (1973) reported that reducing conditions as indicated by declining Eh values, became most favourable for dissolution of Fe near the soil surface. However, the concentration of Fe^{2+} was lowest near the surface because of their leaching from this zone and the shorter time of contact between soil solution and soil particles. The concentration of Fe^{2+} and Mn^{2+} were higher in the deeper horizons of the soil profile.

Gotoh and Patrick (1974) concluded that the distribution between water soluble and exchangeable iron fractions was highly pH dependent with a decrease in pH at a given redox potential increasing the relative amount of ferrous iron in the soil solution is at the expense of that on the exchange complex.

Babarai and Patel (1980) showed three fold increase in available iron when moisture status of the soil was raised from 50 per cent of field capacity to saturation.

Suthar and Patel (1992) reported that Fe, Mn and Zn contents increased significantly with increase in the levels of soil water but decreased pod yield. Similar results found by Sarkar (2000).

Singh *et al.* (1995) reported that excess irrigation of groundnut grown in calcareous soil at Junagadh, exacerbated chlorosis, causing reductions of leaf chlorophyll contents, plant height, dry matter production, pod yield and haulm yields and nutrient uptake.

2.3.2 Plant factors

Olsen and Brown (1980) reported that mobilization of iron at the root-iron oxide interface seems to be achieved by either pH reduction or a drop in redox potential or both, depending upon plant species and variety.

In an experiment conducted by Gowda *et al.* (1993) five iron inefficient (TMV-2, Dh-3-30, JL-24, KRG-1 and S-204) and three efficient (GG-2, Dh-8 and Tatu) genotypes and their F_1 progeny were grown in calcareous soil during summer 1989 at Dharwad and parents and F_2 progeny were grown during *kharif* 1990.

F_1 hybrids from all the crosses were inefficient, indicating that absorption efficiency is dominant and inefficiency is recessive. F_2 progeny from crosses involving GG-2 and Dh-8 segregated 21 efficient; 43 inefficient plants, indicating the involvement of genes, designated as I-Faa and I-Fbb.

To evaluate iron absorption efficiency of groundnut (*Arachis hypogaea* L.) cultivars Kulkarni *et al.* (1994) conducted a pot experiment. They observed for visual chlorosis, Fe^{2+} and chlorophyll content. Based on these parameters they also reported that Tatu, GG-2 and Dh-8 were iron efficient plants among 8 cultivars selected for screening.

Reddy *et al.* (1993) studied differential response of groundnut genotypes to iron deficiency stress in soil containing 4.5 per cent $CaCO_3$ in pots. They reported that total chlorophyll followed by Fe^{2+} were the most sensitive parameters to Fe deficiency. Based on the visual deficiency symptoms (chlorosis score) the genotypes were classified into three groups; Fe efficient (no genotype was found efficient); moderately Fe efficient (TCGS-2, TCGS-3); and Fe inefficient (TCGS-1, TCGS-30 and TMV-2).

Kulkarni *et al.* (1995) conducted a pot culture experiment with calcareous soil at Dharwad where in they had crossed 5 iron inefficient cultivars crossed with three iron efficient genotypes F_1 hybrids were evaluated for iron chlorosis in calcareous and non-calcareous soils. Iron efficient were dark green or green in both calcareous and non-calcareous soils. Whereas, iron-inefficient genotypes were light green to yellow in calcareous soil.

Motagi *et al.* (2000) studied 13 groundnut (*Arachis hypogaea* L.) genotypes for iron deficiency chlorosis in pot experiments and iron efficient were classified based on visual chlorosis rating and chlorophyll content. They classified the cultivars into iron efficient genotypes (GBFDS-272 and Dh-8), moderately efficient genotypes [VL-1, 28-2 and 28-2 (S)] and susceptible cultivar (R-8808).

Goos and Johnson (2000) reported that soybean cultivars (Glacier and council) which are susceptible to iron chlorosis (Fe-inefficient) showed better response to foliar spray of Fe-EDTA in increasing yield than Fe-efficient 'Traill' cultivar.

Nagarathnamma R. (2011) conducted a pot culture experiment to evaluate the groundnut genotypes for lime induced chlorosis tolerance at College of Agriculture, University of Agricultural Sciences, Dharwad. She reported genotypes TMV-2, JL-24 and GPBD-4 recorded significantly higher visual scores on 1 to 5 scale with higher per cent chlorosis and it was least in the DERM and GPBDM genotypes. DERM genotypes, DERM(VLS) had higher peroxidase activity, higher ferrous iron, chlorophyll content and was more iron efficient groundnut genotype as compared to other genotypes.

2.3.3 Nutritional factors

Iyengar (1968) reported that the iron content of the tissue increased in general with increase in the level of iron in the medium but such increase had not always been linear or proportional. The iron content being lower in coffee chlorotic leaves than green leaves when high Mn concentration was present in the medium.

Adverse results about phosphate-iron antogonism were obtained by Mullner (1979) who even at very high values of available 'P' in calcareous soil, found the indications of phosphate induced vine chlorosis.

Venkatasubramanyam and Mehta (1975) at Anand, Gujarat studied on availability of iron in presence of different levels of Zn, Fe and moisture in calcareous soil. They found antigonistic effect of Zn on Fe availability.

Iron deficient leaves are often rich in phosphate (Mangel and Bijbai, 1983). Rao *et al.* (1993) reported strong significant negative correlations (r = -0.90) between ferrous iron and P in plant.

The potassic fertilizer seem to affect iron availability. Shaviv and Hagin (1987) reported that inclusion of K_2SO_4 with $FeSO_4$ has resulted in correction of Fe-chlorosis of peanuts on a highly calcareous soil.

Patel *et al.* (1995) conducted an experiment in the Saurashtra region of Gujarat. From 11 taluks comprising 29 villages both soil and plant samples were collected from chlorotic and healthy plants. They reported that interveinal chlorosis was not due to Fe, S, Zn or Mn deficiency in the soil and it was suggested that chlorosis was caused by an imbalance of nutrients leading to inactivation of P, Ca, Mg, Fe, Zn and Cu particularly in younger plant parts.

2.3.4 Microbial factors

Jurkevitch *et al.* (1988) reported that *Pseudomonas putida* strain isolated from peanut (*Arachis hypogaea* L.) roots excreted yellow-green fluorescent siderophores (pigments) when grown under Fe-deficient condition. In a growth chamber experiment Fe from unpurified Fe-siderophores produced by *Pseudomonas putida* was used on highly calcareous soil at a rate of 11 mg per kg and 19 mg per kg of soil. Groundnut plants grown on these soil were able to produce 75 and 100 per cent concentration, respectively to that of Fe-EDDHA.

Sharwat *et al.* (1990) noticed in a sterilized sand-vermiculite medium supplied with N-free nutrient solution (pH 7.0), inoculation of nodulating groundnut with *Bradyrhizobium* strain NC-43.3 enhanced whole plant dry matter production and O-phenanthroline extractable Fe and N contents of the plants.

2.3.5 Environmental factors

Wei *et al.* (1994) conducted a lab experiment to know influence of soil temperature (from 7 to 33^{0}C) on chlorosis expression and mineral nutrition of Fe deficiency cultivars using ‘Karridale’ (susceptible) and ‘Kaola’ (resistant) subclover varieties on low Fe Parria soil (soil pH 8.2 clayey, hyperthermic, shallow petrocalcic paleustoll). They reported that Karridale was more chlorotic at low and high soil temperature than at intermediate temperature while, the Kaola changed chlorotic expression with soil temperature which indicated the cultivars difference to temperature.

2.4 Correction of iron deficiency

2.4.1 Role of organic acids in correction of iron deficiency

Rogers and Shive (1932) reported that possibility of organic acids maintaining Fe in soluble forms within the plant. This hypothesis was proposed because of Fe^{3+} can form stable, water-soluble complexes with organic acids.

McGeorge (1949) found increased citric acid in Fe-deficient leaves of different plant species. Iljin (1951) reported increased organic acids such as citric acid and malic acid in leaf sap of moderately chlorotic leaves of several plant species. In severely chlorotic leaves, citric acid in leaf sap increased with respect to the green controls, whereas malic acid decreased.

Tiffin (1970) reported that Fe-citrate is the physiologically important Fe compared to translocated in crop species.

Brown and Tiffin (1965) reported that Fe-deficiency increased approximately 3-fold the concentrations of citrate in stem exudate of the Fe-deficient 'Hawkeye' soybean and they also found that the concentrations of citrate in stem exudate of soybean increased considerably upon Fe resupply.

Landsberg (1981) reported that organic acids were proposed to be the source for H^+ released by the roots and also indicated that one of the possible beneficial effects of the increased root citrate under Fe-deficiency could be to control cytoplasmic pH during enhanced proton extrusion.

Mullins *et al.* (1986) reported that most of the Fe (>96%) was predicted to be bound to citrate.

Bienfait and Scheffers (1992) postulated that in plants grown in calcareous soils (that have a high citrate : Fe ratio), citrate photo destruction would lead to formation of ferrous iron. This was derived from experiments carried out *in vitro* with solutions containing 10 µM Fe and 1 µM citrate.

Bruggemann *et al.* (1993) in leaf disks study related to Fe acquisition by mesophyll tissue found that Fe^{3+} reduction is pre-requisite for Fe uptake by leaf disks. This has been confirmed with sunflower leaf disks by Nikolic and Romheld (1999). They reported that Fe reduction process is light dependent and seems capable of using artificial Fe chelates such as Fe^{3+}-EDTA and natural chelates such as Fe(III)-citrate and Fe(III)-malate.

Abadia *et al.* (2002) reported that organic acid concentrations often increases with iron deficiency in different plant parts such as roots, leaves and stem exudates and they summarized data available on the changes in the concentrations of organic anions in plant with iron deficiency and the effect of these changes in plant metabolism.

Abadia *et al.* (2002) in review, summarized that organic acids accumulate in most Fe-deficient plant parts. The pressure of these compounds had an impact on many aspects of the physiology of Fe-deficient plants, including mechanisms directed to facilitate Fe-acquisition by organic acids and proton excretion from roots and to supply the ferric chelate reductase enzymes with enough reducing power. These mechanism could be very important for plants growing in calcareous soils where an absolute Fe-deficiency does not take place and HCO_3^- is always present.

2.4.2 Two principal methods of treating iron deficiency

Larrea (1969) conducted experiment on clayey soils of pH 7.8 to 8.7 and found that chlorosis of beans, soybean, lentil and cowpea was controlled by foliar application of 1.5 to 2.0 per cent solution of $FeSO_4$ at 8 and 16 days after emergence of plants with 40 litres of solution per ha.

Hartzook *et al.* (1971) reported that in irrigated field trails having pH 7.6 to 8.3 and $CaCO_3$ upto 21.40 per cent, the yield of unshelled nuts increased upto 250 per cent by foliar application of 10 kg per ha iron chelates compared with no iron application. Hartzook *et al.* (1972) observed increase in number of pods per plant the average pod and kernel weights and consequently the yield per unit area by the iron chelate treatments.

Jones (1972) reported that application of iron either to soil or to foliage increased the number of flowers, pegs and pods per plant and as a result, increased the yield of groundnut by 15 to 41 per cent. Patil (1977) reported that Fe spray correlated the chlorotic symptoms and pod yield of groundnut increased by 8.2 per cent.

Savitri and Sreeramulu (1980) obtained substantial increase in kernel yield of groundnut by the application of iron. Jurkevitch *et al.* (1986) observed a significant increase in chlorophyll concentration of plants treated with Fe-EDDHA @ 10 mg per 500 g soil after seven weeks of growth in a growth chamber.

Lucena *et al.* (1987) evaluated the iron chelates (Fe-EDTA, Fe-DTPA, Fe-EDDHA and Texene) on a calcareous soil as soil amendments. They reported that in the soil solution Fe was displaced more readily from EDTA chelate and less so from EDDHA chelates.

Singh *et al.* (1990) conducted pot experiment in calcareous soil of Saurashtra to know the effect of different sources of iron on leaf chlorosis, nutrient uptake and yield of groundnut. Application of $FeSO_4$ and Fe-EDTA decreased chlorosis and increased chlorophyll contents of leaves, uptake of Fe and pod yield of groundnut significantly. The foliar spray of 0.5 per cent acqueous solutions of iron sulphate and Fe-EDTA at 20, 35, 50 and 65 days after emergence was more effective than their soil application.

Ramaswami (1992) found that the application of $FeSO_4$ as foliar spray along with 0.1 per cent citric acid influenced on the yield of sugarcane positively though not on the quality parameter. Iron availability was enhanced when pyrite was applied to 300 kg per ha along with mussorie phos as source of P or FYM at 10 t per ha level.

Papastylianou (1990) studied the effectiveness of different Fe chelates and $FeSO_4$ to correct lime induced chlorosis of peanut (*Arachis hypogaea* L.) on calcareous soils in cyprus. He reported that impressive yield response was obtained with Fe chelates, with the degree of response depending on the degree of chlorosis. At a high rate of $FeSO_4$ application there was an increase of yield.

Singh and Dayal (1992) conducted field experiments in 1987 and 1989 at Junagadh. Groundnut cv. Girnar were grown on Fe-deficient calcareous soil. Severe interveinal to complete leaf chlorosis was observed on 38-45 per cent of plant populations during 30 to 60 days after emergence. Foliar application (4 spray of 0.5 kg each at 30, 45, 60 and 75 DAE) of $FeSO_4$, $FeSO_4$ + citric acid, iron citrate and Fe-EDTA produced recoveries of 46, 49, 47 and 46 per cent, respectively greater than control and yields were 13-19, 16-24, 12-18 and 11-18 greater than the control, respectively.

Patel *et al.* (1993) reported that the chlorosis in groundnut grown in calcareous soil was due to inactivation of iron in the plant and it can be cured by foliar spray of soluble salts of iron and found treatment of 1% $FeSO_4$ + 0.1% citric acid and 2% ferric citrate significantly increased pod and haulm yield by about 2 fold through significantly increasing chlorophyll and active iron contents in the leaves by about 3 to 4 fold over control.

Singh *et al.* (1993) conducted a field experiment at Junagadh, Gujarat to study the effect of foliar spray of multimicronutrients and $FeSO_4$ on recovery from chlorosis and yield losses of groundnut and reported that foliar spray of $FeSO_4.7H_2O$ @ 0.5% and multimicronutrients (0.5% $FeSO_4$, 0.2% $ZnSO_4$, 0.2% $MnSO_4$, 0.05% $CuSO_4$, 0.05% H_3BO_3 and 0.01% sodium molybdate) caused regreening of chlorotic leaves, increased chlorophyll contents, pod and haulm yields significantly.

Kundal *et al.* (1994) reported that foliar spray concentration of $FeSO_4.7H_2O$, Fe-EDTA and Fe-citrate below 0.6 per cent on peach root stock seedlings were effective in influencing iron chlorosis, foliar iron and chlorophyll content in seedlings. The spray concentrations from 0.6 per cent onward caused phytotoxicity and subsequent mortality of seedlings at 0.8 and 1.0 per cent application.

Potdar *et al.* (1995) conducted experiment on farm performance of groundnut under different land configurations and foliar iron sprays for the correction of Fe chlorosis on calcareous soil at two village sites prone to Fe chlorosis in Maharashtra. Groundnut crop was established on two land configuration (flatbed or ridge) and foliar spray with two Fe sources ($FeSO_4$ and Fe-EDTA) was applied. Chlorosis rating decreased significantly with ridge planting and foliar Fe sprays and pod, haulm and total dry matter increased significantly in these treatments. Two principal methods of treating iron deficiencies are accepted practice at present. Spraying foliage with inorganic salts and synthetic chelates and soil treatment with inorganic salts and synthetic chelates found to be useful. In addition to above methods planting in ridges in calcareous soil found to be effective in correcting iron chlorosis (Potdar *et al.*, 1995).

Singh *et al.* (1995) reported that application of iron sulphate and iron citrate caused regreening of chlorotic leaves of groundnut grown in calcareous soil and significantly increased leaf chlorophyll contents, plant height, dry matter production, pod and haulm yields and nutrient uptake. The pod yield reduction due to chlorosis was 15.9 to 32.3 per cent.

Singh and Chaudhari (1997) conducted field trails in two consecutive years on groundnut in calcareous soil at Junagadh. Application of 10 kg Fe (soil and foliar) helped in recovering the chlorosis of groundnut leaves and increased the dry matter, pod (19.5%) and oil yields (20.1%) and concentration of nutrients in leaf tissue and their uptake by groundnut.

Rashid *et al.* (1997) conducted a nutrient indexing survey of the chlorosis affected groundnut crop grown in the rainfed Potohar plateau of Pakistan. O-phenanthroline extractable Fe^{2+} concentration in plants decreased with increasing severity of chlorosis. The minimum Fe^{2+} requirement in green plants was estimated to be 40 mg per kg dry matter. The plants containing below 40 mg per kg DM shows chlorotic symptoms and they also reported that foliar spray of 1 per cent, sequestrene (NaFe EDDHA) and 0.5% $FeSO_4$ increased Fe^{2+} concentration in plants and it is also reflected in maximum pod yield over control.

Basavaraj and Uppar (2008) conducted a field experiment during kharif to study the effect of different remedial methods to overcome lime-induced iron chlorosis and to increase the crop yield and to assess the iron uptake and examine the changes in solution and DTPA-Fe in soil during the crop growth periods of groundnut (var. TMV-2) . Foliar application of 0.5 per cent of Fe-DTPA and $FeSO_4.7H_2O$ were applied at flowering, pegging and at pod formation stages.

Foliar treatments resulted in high Fe^{2+} concentration in plants over the critical limit (40 mg Fe^{2+}/kg dry matter on dry weight basis), a level at which plants can be considered as non chlorotic, while Fe^{2+} concentration of plants in the treatments where Fe was applied to soil fell below the critical limit at different growth stages although there was an increase of Fe^{2+} compared of RDF alone.

3. MATERIAL AND METHODS

The details of materials used and methodology adopted in the field experiment are presented in this chapter.

3.1 Location of field experiment

A field experiment was conducted in a calcareous Vertisol, which belongs to a farmer at Aminbhavi village in Dharwad (zone 8) during *rabi* 2011.

3.1.1 Basic characterization of soil

The soil of the experiment site was calcareous Vertisol. A composite soil sample from 0-30 cm depth was collected from experimental site before initiating the sowing and analysed for chemical characteristics by employing standard methods (Table. 1).

3.1.1.1 Soil pH

Soil pH was determined in 1:2.5 soil water suspension using a pH meter (Jackson, 1967).

3.1.1.2 Electrical conductivity

Electrical conductivity was determined using conductivity meter in the supernatant solution used for pH measurement (Jackson, 1967).

3.1.1.3 Organic carbon

Organic carbon was estimated by Walkey and Black wet oxidation method by knowing amount of chromic acid used to oxidize the organic matter in finely ground soil using sulphuric acid as a source of heat through heat of dilution and by titrating the unused chromic acid against ferrous ammonium sulphate using diphenylamine as indicator (Jackson 1967).

3.1.1.4 CEC of soil

The adsorbed cations on the exchange complex are displaced with sodium acetate (NaOAc) by leaching the soil with NaOAc solution. The excess of NaOAc was removed by washing the soil with isopropyl alcohol. Further, the adsorbed sodium on the soil was replaced by treating the soil with neutral normal ammonium acetate (NH_4OAc) and the displaced Na in the leachate was measured by flame photometer. The amount of Na measured was an index of CEC of the soil.

3.1.1.5 Free calcium carbonate

Free calcium carbonate content of soil was determined by acid neutralization method as described by Piper (1966).

3.1.2 Available nutrients

3.1.2.1 Nitrogen

Available nitrogen of soil was determined by alkaline potassium permanganate distillation method as described by Subbaiah and Asija (1956).

3.1.2.2 Phosphorus

Available phosphorus content of soil was determined by Olsen's method as outlined by Jackson (1967).

3.1.2.3 Potassium

Available potassium content of the soil was determined flame photometrically after extracting the soil with neutral normal ammonium acetate as described by Jackson (1967).

3.1.2.4 Bicarbonate

Soil to water (1:2.5) extract was used for determination of bicarbonates. Methyl orange was used during bicarbonate neutralization with sulphuric acid (Jackson, 1967).

Table 1: Basic characteristics of soil

Sl. No.	Particulars	Values
1	Soil pH (1:2.5 soil water suspension)	8.02
2	Soil EC (1:2.5 soil water extract) (dS m^{-1})	0.14
3	Organic carbon (g kg^{-1})	6.0
4	Cation exchange capacity (cmol (p+) kg^{-1})	52.50
5	Free calcium carbonate (%)	19.5
6	Available nitrogen (kg ha^{-1})	196
7	Available phosphorus as P (kg ha^{-1})	14.9
8	Available potassium as K_2O (kg ha^{-1})	540
9	DTPA-Fe (ppm)	1.11
10	Solution iron (ppm)	0.62
11	Bicarbonate ion (ppm)	152.54

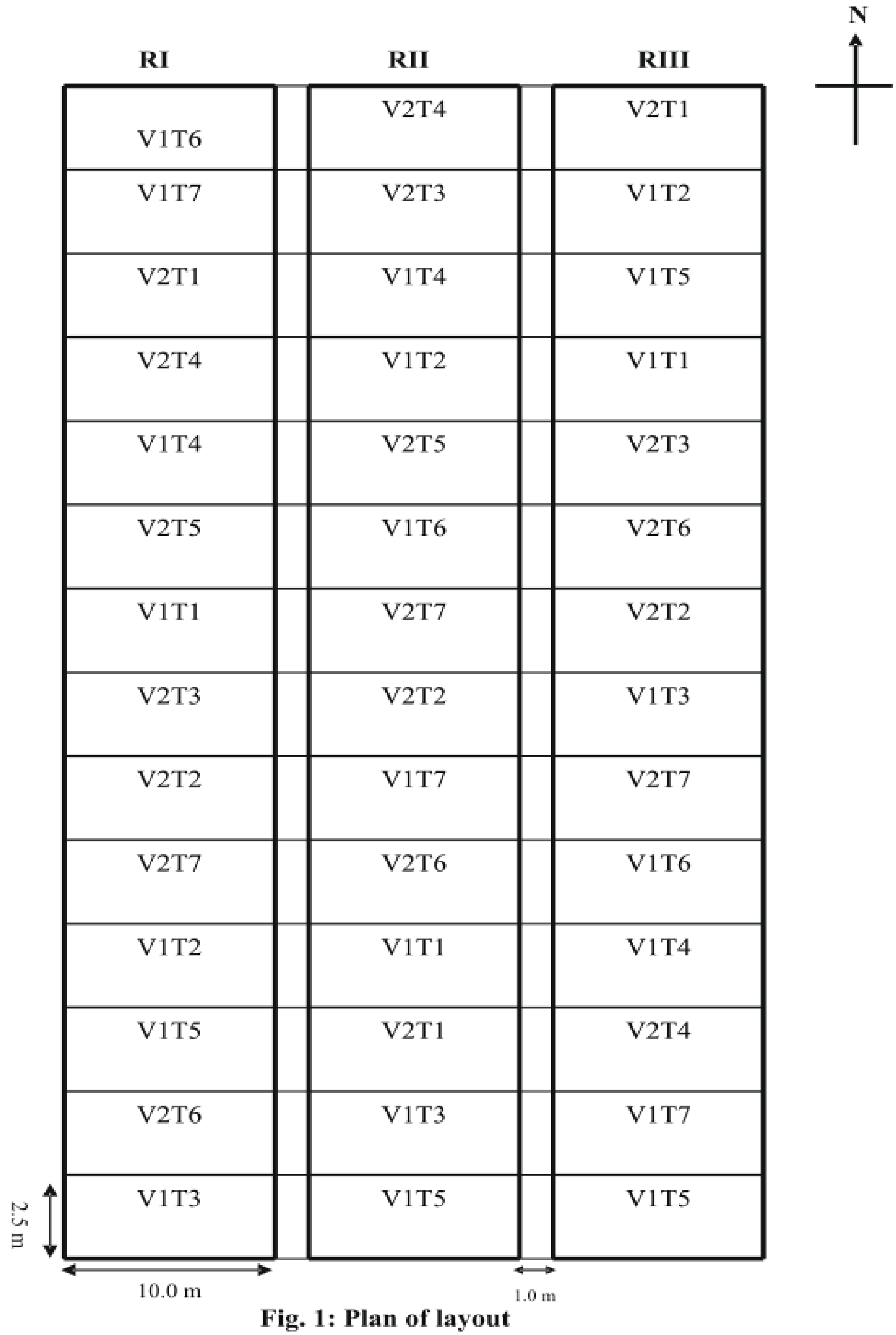

Fig. 1. Plan of layout

Plate 1: General view of the experimental plot at Amminabhavi village of Dharwad taluk

Plate 1. General view of the experimental plot at Amminbhavi village of Dharwad taluk

3.2 Estimation of available forms of iron

3.2.1 Water soluble iron or solution iron

Soil was extracted with water (double distil) maintaining soil to water ratio of 1:40 and equilibrating for 16 hours by shaking on a mechanical shaker. Iron in the extract was determined by AAS (Miller *et al*., 1986).

3.2.2 DTPA Extractable iron

DTPA (Diethylene Triamine Penta Acetic Acid) was used as extractant and AAS was used for estimation as described by Tandon (1998). Five gm of soil was weighed and 20 ml of DTPA reagent was added and kept for shaking about two hours. The filtrate obtained was used for estimation.

3.3 Field experiments details

3.3.1 Design and layout

The field experiment was laid out in a factorial randomized block design with seven treatments and replicated three times. The plan of layout of the experiment is shown in Fig. 1.

3.3.2 Treatments

T_1- Control (with out Fe amendments)

T_2- $FeSO_4.7H_2O$ @ 0.25%

T_3- Citric acid @ 0.25%

T_4- Acetic acid @ 0.25%

T_5- Oxalic acid @ 0.125%

T_6- Lime (citric acid) @ 0.25%

T_7- Hydroxyl amine hydrochloride @ 0.25%

Organic acids were applied as foliar spray at peak flowering, pegging and pod formation stages of crop growth.

Plot size	: 11 m x 3.0 m (gross)
	: 10 m x 2.5 m (net)
Seed rate	: 150 kg ha^{-1}
Varieties	: GPBD-4 and TMV-2
Date of sowing	: 24-11-2011
RDF	: 25:75:25 N, P_2O_5, K_2O kg ha^{-1}

The experiment plot was kept clean and weeds free throughout the crop growth. All cultivation practices were followed as per recommendations.

3.4 Observation on growth characters

Five plants per plot were selected at random for taking observations at different stages.

3.4.1 Visual Chlorosis Rating

The leaves of groundnut showed chlorosis symptoms after 15 days of seedling emergence, which were rated for the severity of leaf chlorosis by determining the per cent of visual chlorosis rating (VCR) scores at after 10 days of foliar spray.

$$\text{Per cent chlorosis} = \frac{\text{Number of chlorotic leaves}}{\text{Number of total leaves}} \times 100$$

The VCR of top five leaves of groundnut was determined using 1 to 5 scale; 1 = no chlorosis (normal green leaves), 2 = slightly chlorosis on few leaves (1-25% chlorosis), 3 = moderate chlorosis on several leaves (26-50% chlorosis) and 5 = severe chlorosis on all leaves (76-100% chlorosis) showing yellow-white colour with necrotic spots.

3.4.2 Plant height

The plant height was measured from the base of the plant to the growing tip and the mean of five plants was taken from the randomly selected five plants in each treatment and expressed in centimeter.

3.4.3 Number of pods per plant

Total number of pods produced was counted from the five randomly selected plants after harvest and their average was taken as the number of pods per plant.

3.4.4 Haulm yield and pod yield

As soon as crop growth period was over, plant samples were collected, washed with water followed by 0.1 N HCl and distilled water and kept for air drying. Air dried haulm samples were oven dried at 70^0C for overnight. The weight of oven dried haulm and air dried pod samples were recorded.

3.5 Plant analysis

3.5.1 Preparation of plant sample for estimation of unassimilated (Fe^{2+}) iron

As soon as fresh leaves of the plant were collected, the leaves were washed with running tap water, followed by 0.1 N HCl and washing with distilled water. The samples were freed off the sticking water drops by sandwiching them between sheets of clean blotting paper.

3.5.2 Extraction and estimation of ferrous iron and total iron

Weighed two gram of fresh chopped leaf samples and transferred to glass bottles. Twenty ml of O-phenanthroline solution were added and the contents of the bottles were stirred gently in order to embathe the plant samples with the extractant. The bottles were stoppered and allowed to stand for about 16 hours at room temperature. The contents were filtered through whatman No. 1 filter paper. Fe^{2+} and total iron were estimated directly in the filterate by measuring the absorbance in spectrophotometer at 510 nm and by AAS, respectively.

3.5.3 PH of cell sap

Cell sap was extracted from the leaf sample and the pH of the sap was estimated using pH meter.

3.5.4 Estimation of total chlorophyll content

Total chlorophyll content was estimated by non destructive method. Hundred mg of fresh leaf tissue was weighed and made it into small pieces. The leaf sample was incubated in 7.0 ml of dimethyl sulfoxide (DMSO) at 65^0 C for 30 minutes. At the end of the incubation period, decanted the supernatant and discarded the leaf tissue. The volume was made up to 10 ml with DMSO. The absorbance of extract was read at 652 nm using DMSO as blank (Shoaf and Lium 1976).

3.6 Statistical analysis

Factorial Randomized Block Design (two factors) was followed for the field experiment. The computed data was interpreted with critical differences level at 5 per cent.

4. EXPERIMENTAL RESULTS

A field experiment was conducted during the *rabi* season of 2011-12 to know the role of organic acids in amelioration of iron chlorosis of two groundnut varieties (var. GPBD-4 and TMV-2) in calcareous Vertisol. The results of the experiment were presented below under different heads.

4.1 Field experiment

4.1.1 Plant analysis before spraying

Leaf samples of two varieties from each treatment were analyzed for Fe^{2+}, Fe^{3+}, total iron, chlorophyll concentration and also pH of cell sap. In addition observations were made regarding visual chlorotic rating.

4.1.1.1 Ferrous (Fe^{2+}) iron concentration

Ferrous (Fe^{2+}) iron concentration was significantly influenced by two varieties of groundnut and was non-significant between the treatments and their interaction (Table 2).

GPBD-4 variety recorded significantly higher ferrous (Fe^{2+}) iron concentration of 45.20 ppm than TMV-2 which recorded 37.75 ppm.

4.1.1.2 Ferric (Fe^{3+}) iron concentration

Ferric (Fe^{3+}) iron concentration was significantly influenced by the two varieties of groundnut and was non-significant between the treatments and interaction between variety and treatment (Table 3).

TMV-2 recorded high ferric concentration of 434.44 ppm than GPBD-4 variety which recorded 426.61 ppm.

4.1.1.3 Total iron concentration

The total iron concentration did not differ significantly with treatments and varieties (Table 4).

However, the GPBD-4 and TMV-2 recorded total iron concentration of 471.81 and 472.19 respectively.

4.1.1.4 Total chlorophyll concentration

The chlorophyll concentration differed significantly between varieties and did not differ significantly with respect to treatments and interaction between variety and treatment (Table 5).

GPBD-4 recorded higher chlorophyll concentration of 1.236 than TMV-2 which recorded 0.983 mg/g on fresh weight basis.

4.1.1.5 pH of the cell sap

pH of the cell sap differed significantly between the varieties and did not differ significantly with respect to treatments and their interaction (Table 6).

The pH values observed in cell sap of GPBD-4 and TMV-2 were 6.53 and 6.64 respectively.

4.1.1.6 Visual chlorosis rating (VCR)

Visual chlorosis rating (VCR) was significantly influenced by two varieties of groundnut crop, but the treatments and interaction between variety and treatment did not differed significantly (Table 7).

Visual chlorisis rating of TMV-2 was 68.95 per cent which comes under scale 4 and GPBD-4 was 37.62 per cent which comes under scale 3.

Table 2: Ferrous iron concentration in groundnut leaves before spraying of organic acids

Treatments	Ferrous iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	43.52	36.96	40.24
T_2 -$FeSO_4.7H_2O$ @ 0.25%	45.68	38.67	42.18 (4.81)*
T_3 -Citric acid @ 0.25%	45.57	38.01	41.79 (3.86)
T_4 -Acetic acid @ 0.25%	45.57	36.99	41.28 (2.59)
T_5 -Oxalic acid @ 0.125%	45.36	36.62	40.99 (1.86)
T_6 -Lime (citric acid) @ 0.25%	46.00	38.38	42.19 (4.85)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	44.11	38.32	41.22 (2.43)
Mean	45.20 (19.64)	37.75	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.36	1.05
Treatment (T)	0.68	NS
V × T	0.96	NS

- Figures in parenthesis indicate percentage change over control

Table 3: Ferric iron concentration in groundnut leaves before spraying of organic acids

Treatments	Ferric iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	425.25	430.12	427.69
T_2 -$FeSO_4.7H_2O$ @ 0.25%	424.59	443.51	434.05 (1.49)*
T_3 -Citric acid @ 0.25%	427.46	429.64	428.5 (0.205)
T_4 -Acetic acid @ 0.25%	427.48	436.04	431.76 (0.95)
T_5 -Oxalic acid @ 0.125%	421.64	433.07	427.30 (-0.85)
T_6 -Lime (citric acid) @ 0.25%	428.93	434.00	431.80 (8.47)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	430.94	434.71	432.82 (1.20)
Mean	426.61 (-1.80)	434.44	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.84	5.36
Treatment (T)	3.45	NS
V × T	4.88	NS

* - Figures in parenthesis indicate percentage change over control

Table 4: Total iron concentration in groundnut leaves before spraying of organic acids

Treatments	Total iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	468.47	467.05	467.76
T_2 -$FeSO_4.7H_2O$ @ 0.25%	470.37	484.48	477.43 (2.07)*
T_3 -Citric acid @ 0.25%	473.03	463.65	468.34 (0.12)
T_4 -Acetic acid @ 0.25%	473.05	473.03	473.04 (1.13)
T_5 -Oxalic acid @ 0.125%	467.00	469.68	468.31 (0.24)
T_6 -Lime (citric acid) @ 0.25%	475.73	472.38	474.06 (1.35)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	475.05	473.03	474.04 (1.34)
Mean	471.81 (-0.02)	472.19	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.82	NS
Treatment (T)	3.41	NS
V × T	4.82	NS

Figures in parenthesis indicate percentage change over control

Table 5: Chlorophyll concentration in groundnut leaves before spraying of organic acids

Treatments	Chlorophyll concentration (mg/g fr.wt.)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	1.244	0.981	1.113
T_2 -$FeSO_4.7H_2O$ @ 0.25%	1.245	1.002	1.124 (0.96)*
T_3 -Citric acid @ 0.25%	1.252	0.990	1.121 (0.70)
T_4 -Acetic acid @ 0.25%	1.223	1.006	1.115 (0.15)
T_5 -Oxalic acid @ 0.125%	1.226	0.968	1.097 (-1.44)
T_6 -Lime (citric acid) @ 0.25%	1.217	0.965	1.091 (-1.98)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	1.243	0.969	1.106 (-0.64)
Mean	1.236 (25.71)	0.983	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.01	0.03
Treatment (T)	0.02	NS
V × T	0.03	NS

* - Figures in parenthesis indicate percentage change over control

Table 6: pH of cell sap of groundnut leaves before spraying of organic acids

Treatments	pH of cell sap		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	6.54	6.64	6.59
T_2 -$FeSO_4.7H_2O$ @ 0.25%	6.53	6.64	6.58 (-0.13)*
T_3 -Citric acid @ 0.25%	6.53	6.64	6.58 (-0.13)
T_4 -Acetic acid @ 0.25%	6.54	6.65	6.59 (0.00)
T_5 -Oxalic acid @ 0.125%	6.53	6.64	6.58 (-0.13)
T_6 -Lime (citric acid) @ 0.25%	6.53	6.65	6.59 (0.00)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	6.53	6.64	6.59 (0.00)
Mean	6.53 (-1.64)	6.64	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.002	0.01
Treatment (T)	0.003	NS
V × T	0.005	NS

* - Figures in parenthesis indicate percentage change over control

Table 7: Visual Chlorotic Ratings of groundnut leaves before spraying of organic acids

Treatments	Visual Chlorotic Ratings (%)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	38.00	69.33	53.67
T_2 -$FeSO_4.7H_2O$ @ 0.25%	38.33	67.67	53.00 (-1.25)*
T_3 -Citric acid @ 0.25%	36.33	70.00	53.17 (-0.94)
T_4 -Acetic acid @ 0.25%	37.00	69.33	53.17 (-0.94)
T_5 -Oxalic acid @ 0.125%	37.67	69.33	53.50 (-0.32)
T_6 -Lime (citric acid) @ 0.25%	38.00	68.33	53.17 (-0.94)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	38.00	68.67	53.33 (-0.63)
Mean	37.62 (-45.44)	68.95	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.51	1.49
Treatment (T)	0.96	NS
V × T	1.36	NS

Figures in parenthesis indicate percentage change over control

Table 8: Effect of different organic acids on ferrous iron concentration of groundnut leaves

Treatments	Ferrous iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	39.18	33.14	36.16
T_2-$FeSO_4.7H_2O$ @ 0.25%	39.12	40.05	39.59 (9.47)*
T_3-Citric acid @ 0.25%	38.49	39.07	38.78 (7.24)
T_4-Acetic acid @ 0.25%	35.20	35.05	35.13 (-2.86)
T_5-Oxalic acid @ 0.125%	37.93	35.26	36.59 (1.20)
T_6-Lime (citric acid) @ 0.25%	36.99	39.16	38.07 (5.29)
T_7-Hydroxyl amine hydrochloride @ 0.25%	36.83	37.03	36.93 (2.12)
Mean	37.67 (1.90)	36.97	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.23	0.68
Treatment (T)	0.44	1.28
V × T	0.62	1.80

* - Figures in parenthesis indicate percentage change over control

4.1.2 Plant analysis after spraying

After spraying of organic acids, ferrous iron, ferric iron, total iron concentration, chlorophyll concentration and pH of cell sap were observed. In addition to this visual chlorotic rating were recorded.

4.1.2.1 Ferrous (Fe^{2+}) iron concentration

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different variety, treatments and also by their interactions (Table 8).

GPBD-4 recorded significantly higher ferrous iron concentration of 37.67 ppm compared to TMV-2 variety which recorded 36.97 ppm. Among the treatments, the treatment receiving $FeSO_4.7H_2O$ @ 0.25% was recorded higher ferrous iron concentration of 39.59 ppm and was on par with the treatment receiving citric acid @ 0.25% which recorded ferrous iron concentration of 38.78 ppm.

4.1.2.2 Ferric (Fe^{3+}) iron concentration

Ferric iron concentration was significantly influenced by two varieties, treatments and their interactions.

TMV-2 variety significantly recorded higher ferric iron concentration of 319.22, whereas GPBD-4 variety recorded 295.07 ppm. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher ferric iron concentration (366.26 ppm), in the different treatments, treatments receiving $FeSO_4.7H_2O$ @ 0.25% (306.47) and acetic acid @ 0.25% (306.48) were on par with one another (Table 9).

4.1.2.3 Total Fe concentration

Total iron concentration after spraying was significantly influenced by different treatments, varieties and their interaction.

TMV-2 variety recorded significantly higher total iron concentration of 356.29 ppm, whereas GPBD-4 variety recorded 332.75 ppm. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had high total iron concentration (404.33 ppm). However, treatment receiving $FeSO_4.7H_2O$ @ 0.25% (346.05 ppm) with acetic acid @ 0.25% (341.60), and treatment receiving acetic acid @ 0.25% (341.60 ppm) with oxalic acid @ 0.125% (335.79 ppm) were on par with one another (Table 10).

4.1.2.4 Total chlorophyll concentration

Chlorophyll concentration differed significantly between the varieties, treatments and their interaction (Table 11).

GPBD-4 recorded significantly higher chlorophyll concentration of 1.114 mg/g fresh weight compared to TMV-2 which recorded 1.008 mg/g fresh weight. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% recorded higher chlorophyll concentration of 1.320 mg/g fresh weight. However, the treatments receiving lime (citric acid) @ 0.25% and hydroxyl amine hydrochloride @ 0.25% were on par with one another.

4.1.2.5 pH of the cell sap

pH of the cell sap differed significantly between the varieties, treatments and their interaction (Table 12).

GPBD-4 recorded pH value of 6.34 whereas TMV-2 recorded pH value of 6.45. Among the treatments, lowest pH (6.34) was recorded in treatment receiving acetic acid @ 0.25%, however treatments receiving citric acid @ 0.25% with oxalic acid @ 0.125%, $FeSO_4.7H_2O$ @ 0.25% with lime (citric acid) @ 0.25% and $FeSO_4.7H_2O$ @ 0.25% with hydroxyl amine hydrochloride @ 0.25% were on par with one another.

4.1.2.6 Visual chlorosis rating (VCR)

Visual chlorosis rating (VCR) was significantly influenced by variety, treatments and their interaction (Table 13).

Table 9: Effect of different organic acids on ferric iron concentration of groundnut leaves

Treatments	Ferric iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	332.76	254.90	293.83
T_2 -$FeSO_4.7H_2O$ @ 0.25%	355.63	257.30	306.47 (4.30)*
T_3 -Citric acid @ 0.25%	249.75	250.60	250.17 (-14.86)
T_4 -Acetic acid @ 0.25%	262.49	350.46	306.48 (4.30)
T_5 -Oxalic acid @ 0.125%	215.21	383.19	299.20 (1.83)
T_6 -Lime (citric acid) @ 0.25%	328.81	403.71	366.26 (24.65)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	320.87	335.10	327.99 (11.63)
Mean	295.07 (-7.59)	319.32	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.27	3.69
Treatment (T)	2.37	6.90
V × T	3.36	9.75

* - Figures in parenthesis indicate percentage change over control

Table 10: Effect of different organic acids on total iron concentration of groundnut leaves

Treatments	Total iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	371.93	288.03	329.98
T_2 -$FeSO_4.7H_2O$ @ 0.25%	394.75	297.35	346.05 (4.87)*
T_3 -Citric acid @ 0.25%	288.23	289.67	288.95 (-12.43)
T_4 -Acetic acid @ 0.25%	297.68	385.52	341.60 (3.52)
T_5 -Oxalic acid @ 0.125%	253.13	418.45	335.79 (1.76)
T_6 -Lime (citric acid) @ 0.25%	365.80	442.87	404.33 (22.53)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	357.70	372.13	364.92 (10.59)
Mean	332.75 (-6.61)	356.29	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.39	4.05
Treatment (T)	2.61	7.57
V × T	3.68	10.71

* - Figures in parenthesis indicate percentage change over control

Table 11: Effect of different organic acids on chlorophyll concentration of groundnut leaves

Treatments	Chlorophyll concentration (mg/g fr.wt.)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	0.957	0.851	0.904
T_2 -$FeSO_4.7H_2O$ @ 0.25%	1.356	1.284	1.320 (46.02)*
T_3 -Citric acid @ 0.25%	1.278	1.013	1.145 (26.68)
T_4 -Acetic acid @ 0.25%	1.345	1.167	1.256 (38.94)
T_5 -Oxalic acid @ 0.125%	0.909	0.874	0.892 (-1.35)
T_6 -Lime (citric acid) @ 0.25%	0.978	0.955	0.966 (6.88)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	0.977	0.916	0.947 4.70)
Mean	1.114 (10.54)	1.008	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.01	0.02
Treatment (T)	0.01	0.04
V × T	0.02	0.05

* - Figures in parenthesis indicate percentage change over control

Table 12: Effect of different organic acids on pH of cell sap of groundnut leaves

Treatments	pH of cell sap		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	6.53	6.64	6.58
T_2 -$FeSO_4.7H_2O$ @ 0.25%	6.33	6.44	6.39 (-2.96)*
T_3 -Citric acid @ 0.25%	6.31	6.40	6.36 (-3.39)
T_4 -Acetic acid @ 0.25%	6.29	6.38	6.34 (-3.70)
T_5 -Oxalic acid @ 0.125%	6.29	6.42	6.35 (-3.44)
T_6 -Lime (citric acid) @ 0.25%	6.32	6.44	6.38 (-3.01)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	6.34	6.45	6.40 (-2.81)
Mean	6.34 (-1.64)	6.45	

Comparison of	S.Em±	CD (P=0.05)
Variety A)	0.002	0.01
Treatment B)	0.004	0.01
A × B	0.01	0.02

* - Figures in parenthesis indicate percentage change over control

Table 13: Effect of different organic acids on Visual Chlorotic Ratings of groundnut leaves

Treatments	Visual Chlorotic Ratings (%)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	41.33	56.33	48.83
T_2-$FeSO_4.7H_2O$ @ 0.25%	19.00	24.33	21.67 (-55.63)*
T_3-Citric acid @ 0.25%	24.33	27.00	25.67 (-47.44)
T_4-Acetic acid @ 0.25%	22.00	28.67	25.33 (-48.12)
T_5-Oxalic acid @ 0.125%	25.00	28.67	26.83 (-45.05)
T_6-Lime (citric acid) @ 0.25%	20.00	30.33	25.17 (-48.46)
T_7-Hydroxyl amine hydrochloride @ 0.25%	22.00	35.00	28.50 (-41.63)
Mean	24.81 (-24.59)	32.90	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.20	0.59
Treatment (T)	0.38	1.10
V × T	0.54	1.56

* - Figures in parenthesis indicate percentage change over control

Table 14: Ferrous iron concentration in groundnut leaves at 30 DAS

Treatments	Ferrous iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	33.22	26.99	30.10
T_2 -$FeSO_4.7H_2O$ @ 0.25%	34.57	28.52	31.55 (4.80)*
T_3 -Citric acid @ 0.25%	34.45	27.32	30.89 (2.62)
T_4 -Acetic acid @ 0.25%	35.43	27.29	31.36 (4.20)
T_5 -Oxalic acid @ 0.125%	35.34	26.86	31.10 (3.33)
T_6 -Lime (citric acid) @ 0.25%	35.91	27.85	31.88 (5.91)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	34.05	27.75	30.90 (2.65)
Mean	34.78 (25.86)	27.58	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.33	0.96
Treatment (T)	0.62	NS
V × T	0.88	NS

* - Figures in parenthesis indicate percentage change over control

DAS – Days after sowing

GPBD-4 has low VCR rating of 24.81 per cent that comes under scale 2, whereas TMV-2 recorded VCR rating of 32.90 per cent which comes under scale 3. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% was shown less reading of 21.67 per cent. However, treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @ 0.25% were on par with one another.

4.1.3 Plant analysis at 30, 60 DAS and at harvest

4.1.3.1 Ferrous (Fe^{2+}) iron concentration at 30 DAS

Ferrous (Fe^{2+}) iron concentration was significantly influenced by two varieties of groundnut and was non-significant between the treatments and their interaction (Table 14).

GPBD-4 variety recorded significantly higher ferrous (Fe^{2+}) iron concentration of 34.78 ppm than TMV-2 which reecorded 27.58 ppm.

4.1.3.2 Ferric (Fe^{3+}) iron concentration at 30 DAS

Ferric (Fe^{3+}) iron concentration was significantly influenced by the two varieties of groundnut and was non-significant between the treatments and interaction between variety and treatment (Table 15).

TMV-2 recorded higher ferric iron concentration of 423.53 ppm than GPBD-4 variety which recorded 417.43 ppm.

4.1.3.3 Total iron concentration at 30 DAS

The total iron concentration did not differ significantly with treatments and varieties (Table 16).

However, the GPBD-4 and TMV-2 recorded total iron concentration of 452.21 and 451.11 respectively.

4.1.3.4 Ferrous (Fe^{2+}) iron concentration at 60 DAS

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different variety, treatments and also by their interactions.

GPBD-4 recorded significantly higher ferrous iron concentration of 46.00 ppm compared to TMV-2 variety which recorded 38.34 ppm. Among the treatments, the treatment receiving $FeSO_4.7H_2O$ @ 0.25% recorded higher ferrous iron concentration of 43.28 ppm and was on par with the treatment receiving citric acid @ 0.25% which recorded ferrous iron concentration of 43.24 ppm (Table 17).

4.1.3.5 Ferric (Fe^{3+}) iron concentration at 60 DAS

Ferric iron concentration was significantly influenced by different variety, treatments and their interactions.

TMV-2 variety recorded significantly higher ferric iron concentration of 288.50 ppm, whereas GPBD-4 variety recorded 256.48 ppm. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher ferric iron concentration (330.47 ppm), in different treatments, treatments receiving $FeSO_4.7H_2O$ @ 0.25% (264.71 ppm) and acetic acid @ 0.25% (268.57) were on par with one another (Table 18).

4.1.3.6 Total Fe concentration at 60 DAS

Total iron concentration after spraying was significantly influenced by different treatments, varieties and their interaction.

TMV-2 variety recorded significantly higher total iron concentration of 326.84 ppm, whereas GPBD-4 variety recorded 302.49 ppm. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher total iron concentration (373.99). However, treatment receiving $FeSO_4.7H_2O$ @ 0.25% (307.99) and acetic acid @ 0.25% (307.26), acetic acid @ 0.25% (311.05) and oxalic acid @ 0.125% (307.26) were on par with one another (Table 19).

4.1.3.7 Ferrous (Fe^{2+}) iron concentration at harvest

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different varieties, treatments and also by their interaction (Table 20).

Table 15: Ferric iron concentration in groundnut leaves at 30 DAS

Treatments	Ferric iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	420.33	423.40	421.87
T_2-$FeSO_4.7H_2O$ @ 0.25%	419.10	421.32	420.21 (-0.39)*
T_3-Citric acid @ 0.25%	418.91	425.03	421.97 (0.02)
T_4-Acetic acid @ 0.25%	416.97	422.59	419.78 (-0.50)
T_5-Oxalic acid @ 0.125%	415.06	426.16	420.61 (-0.30)
T_6-Lime (citric acid) @ 0.25%	415.06	424.12	419.59 (-0.54)
T_7-Hydroxyl amine hydrochloride @ 0.25%	416.58	422.08	419.33 (-0.60)
Mean	417.43 (-1.44)	423.53	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.98	2.85
Treatment (T)	1.83	NS
V × T	2.59	NS

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

Table 16: Total iron concentration in groundnut leaves at 30 DAS

Treatments	Total iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	453.55	450.38	451.97
T_2 -$FeSO_4.7H_2O$ @ 0.25%	453.67	449.84	451.76 (-0.05)*
T_3 -Citric acid @ 0.25%	453.37	452.35	452.86 (0.20)
T_4 -Acetic acid @ 0.25%	452.40	449.89	451.14 (-0.18)
T_5 -Oxalic acid @ 0.125%	450.41	453.02	451.71 (-0.06)
T_6 -Lime (citric acid) @ 0.25%	451.47	452.47	451.97 (0.00)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	450.63	449.82	450.23 (-0.39)
Mean	452.21 (0.24)	451.11	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.96	NS
Treatment (T)	1.80	NS
V × T	2.54	NS

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

Table 17: Ferrous iron concentration in groundnut leaves at 60 DAS

Treatments	Ferrous iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	38.51	37.03	37.77
T_2 -$FeSO_4.7H_2O$ @ 0.25%	46.91	39.65	43.28 (14.60)*
T_3 -Citric acid @ 0.25%	47.00	39.57	43.29 (14.60)
T_4 -Acetic acid @ 0.25%	47.57	37.39	42.48 (12.47)
T_5 -Oxalic acid @ 0.125%	47.48	37.23	42.36 (12.14)
T_6 -Lime (citric acid) @ 0.25%	48.11	38.93	43.52 (15.22)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	46.50	38.61	42.56 (12.68)
Mean	46.01 (20.01)	38.34	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.28	0.81
Treatment (T)	0.52	1.51
V × T	0.74	2.15

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

Table 18: Ferric iron concentration in groundnut leaves at 60 DAS

Treatments	Ferric iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	313.18	221.01	267.10
T_2 -$FeSO_4.7H_2O$ @ 0.25%	301.59	227.82	264.71 (-0.90)*
T_3 -Citric acid @ 0.25%	214.03	219.43	216.73 (-18.86)
T_4 -Acetic acid @ 0.25%	218.99	318.15	268.57 (0.55)
T_5 -Oxalic acid @ 0.125%	177.95	351.86	264.91 (-0.82)
T_6 -Lime (citric acid) @ 0.25%	287.25	373.69	330.47 (23.73)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	282.38	307.50	294.94 (10.42)
Mean	46.01 (-10.53)	38.34	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.12	3.26
Treatment (T)	2.09	6.08
V × T	2.96	8.60

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

Table 19: Total iron concentration in groundnut leaves at 60 DAS

Treatments	Total iron concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	351.69	258.04	304.87
T_2 -$FeSO_4.7H_2O$ @ 0.25%	348.50	267.47	307.99 (1.02)*
T_3 -Citric acid @ 0.25%	261.03	259.00	260.02 (-14.71)
T_4 -Acetic acid @ 0.25%	266.57	355.54	311.05 (2.03)
T_5 -Oxalic acid @ 0.125%	225.44	389.09	307.26 (0.78)
T_6 -Lime (citric acid) @ 0.25%	335.35	412.62	373.99 (22.67)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	328.88	346.11	337.50 (10.70)
Mean	302.49 (-7.45)	326.84	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.12	3.26
Treatment (T)	2.10	6.10
V × T	2.98	8.66

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

Table 20: Ferrous iron concentration in groundnut leaves at harvest

Treatments	Ferrous iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	45.99	42.22	44.10
T_2 -$FeSO_4.7H_2O$ @ 0.25%	44.74	56.41	50.57 (14.68)*
T_3 -Citric acid @ 0.25%	56.35	54.43	55.39 (25.59)
T_4 -Acetic acid @ 0.25%	59.74	62.76	61.25 (38.89)
T_5 -Oxalic acid @ 0.125%	52.72	53.90	53.31 (20.88)
T_6 -Lime (citric acid) @ 0.25%	50.65	57.91	54.28 (23.08)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	64.36	54.53	59.45 (34.80)
Mean	53.51 (-1.98)	54.59	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.30	0.87
Treatment (T)	0.56	1.62
V × T	0.79	2.30

* - Figures in parenthesis indicate percentage change over control

Table 21: Ferric iron concentration in groundnut leaves at harvest

Treatments	Ferric iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	175.63	110.92	143.27
T_2 -$FeSO_4.7H_2O$ @ 0.25%	180.09	128.13	154.11 (7.57)*
T_3 -Citric acid @ 0.25%	200.34	87.61	143.97 (0.49)
T_4 -Acetic acid @ 0.25%	92.96	82.91	87.94 (-38.62)
T_5 -Oxalic acid @ 0.125%	113.92	192.23	153.07 (6.84)
T_6 -Lime (citric acid) @ 0.25%	205.91	50.43	128.17 (-10.54)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	135.19	133.47	134.33 (-6.24)
Mean	157.72 (40.52)	112.24	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.79	2.28
Treatment (T)	1.47	4.27
V × T	2.08	6.04

* - Figures in parenthesis indicate percentage change over control

Table 22: Total iron concentration in groundnut leaves at harvest

Treatments	Total iron concentration (ppm)		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	221.62	153.13	187.38
T_2 -$FeSO_4.7H_2O$ @ 0.25%	224.83	184.53	204.68 (9.23)*
T_3 -Citric acid @ 0.25%	256.68	142.03	199.36 (6.39)
T_4 -Acetic acid @ 0.25%	152.70	145.67	149.18 (-20.38)
T_5 -Oxalic acid @ 0.125%	166.63	246.13	206.38 (10.14)
T_6 -Lime (citric acid) @ 0.25%	256.57	108.33	182.45 (-2.63)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	199.55	188.00	193.78 (3.41)
Mean	211.23 (26.61)	166.83	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.69	2.01
Treatment (T)	1.29	3.76
V × T	1.83	5.32

* - Figures in parenthesis indicate percentage change over control

At harvest TMV-2 variety recorded higher Fe^{2+} iron concentration of 54.59 ppm whereas, GPBD-4 recorded 53.51 ppm. Among the treatments, treatment receiving acetic acid @ 0.25% had higher ferrous iron concentration.

4.1.3.8 Ferric (Fe^{3+}) iron concentration at harvest

Ferric iron concentration was significantly influenced by different varieties, treatments and their interaction.

GPBD-4 variety recorded higher ferric iron concentration of 157.72 ppm, whereas, TMV-2 recorded 112.24 ppm. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% recorded higher concentration of 154.11 ppm. However, it was on par with treatment receiving oxalic acid @ 0.125% (Table 21).

4.1.3.9 Total Fe concentration at harvest

Total iron concentration at harvest was significantly influenced by variety, treatment and their interaction (Table 22).

Total iron concentration recorded in GPBD-4 was 211.23, whereas, in TMV-2 it recorded 166.83 ppm. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% and oxalic acid @ 0.125% were recorded higher total Fe value of 204.68 and 206.38 respectively.

4.2 Soil analysis at harvest

4.2.1 DTPA iron

DTPA iron concentrations at harvest differed significantly with varieties, treatments and their interaction (Table 23).

DTPA iron was 0.428 ppm in variety TMV-2, whereas, in variety GPBD-4 it was 0.402 ppm. Among the treatments, treatment receiving oxalic acid @ 0.125% was recorded high DTPA iron concentration of 0.458 ppm, whereas in control it recorded 0.331 ppm.

4.2.2 Solution iron

Solution iron at harvest differed significantly among the variety, treatments and their interaction (Table 23).

TMV-2 recorded high solution iron of 0.217 ppm, whereas, in GPBD-4 it was recorded 0.211 ppm, among the different treatments, treatment receiving citric acid @ 0.25% recorded higher solution iron concentration of 0.239 ppm, whereas treatments receiving acetic acid @ 0.25%, oxalic acid @ 0.125% and lime (citric acid) @ 0.25% did not differ significantly.

4.2.3 Bicarbonate ion concentration

Bicarbonate ion concentration did not differ significantly between varieties, treatments and in variety-treatment interactions (Table 24). The bicarbonate ion concentration in the soil ranges from 149.68 to 154.53 ppm

4.3 Growth parameters

4.3.1 Plant height

Plant height differed significantly with respect to varieties and did not differ significantly with respect to treatments at 30 DAS, whereas at 60 DAS, Plant height differed significantly with variety and treatments and their interaction (Table 25).

The value of plant height recorded in GPBD-4 at 30 DAS was 6.23 cm, whereas in TMV-2 it recorded 5.67 cm but at 60 DAS the plant height recorded in GPBD-4 was 14.2 cm and in TMV-2 was 13.41 cm. Among the treatments at 60 DAS, the treatment receiving $FeSO_4.7H_2O$ @ 0.25% had higher plant height of 14.98 cm, whereas in control it was 11.50 cm.

Table 23: DTPA and solution iron concentration in soil at harvest

Treatments	DTPA-iron concentration (ppm)			Solution iron concentration (ppm)		
	GPBD-4	TMV-2	Mean	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	0.308	0.354	0.331	0.191	0.185	0.188
T_2 -$FeSO_4.7H_2O$ @ 0.25%	0.383	0.425	0.404 (22.16)*	0.203	0.221	0.212 (12.85)*
T_3 -Citric acid @ 0.25%	0.401	0.437	0.419 (26.54)	0.234	0.243	0.239 (26.86)
T_4 -Acetic acid @ 0.25%	0.413	0.441	0.427 (29.00)	0.214	0.219	0.217 (15.16)
T_5 -Oxalic acid @ 0.125%	0.449	0.467	0.458 (38.42)	0.217	0.223	0.220 (16.93)
T_6 -Lime (citric acid) @ 0.25%	0.435	0.439	0.437 (32.02)	0.218	0.225	0.221 (17.73)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	0.423	0.435	0.429 (29.71)	0.201	0.204	0.203 (7.71)
Mean	0.402 (-6.12)	0.428		0.211 (-2.72)	0.217	
Comparison of	S.Em±	CD (P=0.05)		S.Em±	CD (P=0.05)	
Variety (V)	0.001	0.002		0.001	0.003	
Treatment (T)	0.001	0.004		0.002	0.006	
V × T	0.002	0.01		0.003	0.01	

* - Figures in parenthesis indicate percentage change over control

Table 24: Bicarbonate ion concentration in soil at harvest

Treatments	Bicarbonate ion concentration (ppm)		
	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	154.40	154.53	154.47
T_2 -$FeSO_4.7H_2O$ @ 0.25%	152.53	153.43	152.98 (-0.96)*
T_3 -Citric acid @ 0.25%	149.53	150.47	150.00 (-2.89)
T_4 -Acetic acid @ 0.25%	154.53	154.53	154.53 (0.04)
T_5 -Oxalic acid @ 0.125%	150.47	149.33	149.90 (-2.96)
T_6 -Lime (citric acid) @ 0.25%	148.90	150.47	149.68 (-3.10)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	153.83	150.47	152.15 (-1.50)
Mean	152.03 (0.09)	151.89	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	1.31	NS
Treatment (T)	2.45	NS
V × T	3.46	NS

* - Figures in parenthesis indicate percentage change over control

Table 25: Effect of different organic acids on plant height of groundnut at 30 and 60 DAS

Treatments	Plant height at 30 DAS (cm)			Plant height at 60 DAS (cm)		
	GPBD-4	**TMV-2**	**Mean**	**GPBD-4**	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	6.23	5.70	5.97	12.67	10.33	11.50
T_2 -$FeSO_4.7H_2O$ @ 0.25%	6.23	5.70	5.97 (0.00)*	15.33	14.63	14.98 (30.29)*
T_3 -Citric acid @ 0.25%	6.23	5.70	5.97 (0.00)	14.60	14.10	14.35 (24.78)
T_4 -Acetic acid @ 0.25%	6.27	5.77	6.02 (0.78)	14.17	13.80	13.98 (21.59)
T_5 -Oxalic acid @ 0.125%	6.20	5.60	5.90 (-1.17)	14.50	13.90	14.20 (23.48)
T_6 -Lime (citric acid) @ 0.25%	6.27	5.60	5.93 (-0.61)	14.23	13.53	13.88 (20.72)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	6.20	5.63	5.92 (-0.89)	13.90	13.57	13.73 (19.42)
Mean	6.23 (9.94)	5.67		14.20 (5.89)	13.41	
Comparison of	S.Em±	CD (P=0.05)		S.Em±	CD (P=0.05)	
Variety (V)	0.02	0.07		0.03	0.08	
Treatment (T)	0.04	NS		0.05	0.15	
V × T	0.06	NS		0.07	0.21	

* - Figures in parenthesis indicate percentage change over control
DAS – Days after sowing

4.3.2 Number of pods per plant

Number of pods per plant significantly differed with treatment and varieties (Table 26).

Number of pods in GPBD-4 was 14.07, whereas in TMV-2 it was 13.26, number of pods per plant have increased from 12.07 in control to 16.77 in the plot receiving treatment $FeSO_4.7H_2O$ @ 0.25%.

4.4 Yield parameters

4.4.1 Pod yield

Pod yield was significantly influenced by various treatments and two varieties (Table 27).

Among the varieties GPBD-4 was shown higher pod yield of 27.09 q/ha as compared to 25.63 q/ha in TMV-2. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% was shown higher yield of 30.22 q/ha. Whereas, treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @ 0.25% were on par with one another.

4.4.2 Haulm yield

Haulm yield differed significantly with varieties, treatments and their interaction (Table 27).

Haulm yield was 40.28 q/ha in TMV-2 variety as compared to 27.02 q/ha in GPBD-4, among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% had higher haulm yield of 38.27 q/ha as compared to control which recorded 30.28 q/ha.

Table 26: Effect of different organic acids on number of pods per plant of groundnut

Treatments	Number of pods per plant		
	GPBD-4	**TMV-2**	**Mean**
T_1-Control (without Fe amendments)	13.47	10.67	12.07
T_2 -$FeSO_4.7H_2O$ @ 0.25%	16.47	17.07	16.77 (38.91)*
T_3 -Citric acid @ 0.25%	13.20	15.40	14.30 (18.48)
T_4 -Acetic acid @ 0.25%	14.87	14.07	14.47 (19.86)
T_5 -Oxalic acid @ 0.125%	12.00	12.13	12.07 (-0.03)
T_6 -Lime (citric acid) @ 0.25%	14.47	12.13	13.30 (10.19)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	14.00	11.33	12.67 (4.94)
Mean	14.07 (6.08)	13.26	

Comparison of	S.Em±	CD (P=0.05)
Variety (V)	0.11	0.33
Treatment (T)	0.21	0.61
V × T	0.30	0.86

* - Figures in parenthesis indicate percentage change over control

Table 27: Effect of different organic acids on pod yield and haulm yield of groundnut

Treatments	Pod yield (q/ha)			Haulm yield (q/ha)		
	GPBD-4	TMV-2	Mean	GPBD-4	TMV-2	Mean
T_1-Control (without Fe amendments)	26.45	21.67	24.06	26.80	33.76	30.28
T_2 -$FeSO_4.7H_2O$ @ 0.25%	29.78	30.67	30.22 (25.62)*	30.04	46.49	38.27 (26.38)*
T_3 -Citric acid @ 0.25%	25.56	28.33	26.94 (11.98)	26.98	45.96	36.47 (20.43)
T_4 -Acetic acid @ 0.25%	27.55	26.22	26.89 (11.76)	28.44	42.71	35.58 (17.50)
T_5 -Oxalic acid @ 0.125%	25.99	25.44	25.72 (6.89)	25.60	36.40	31.00 (2.38)
T_6 -Lime (citric acid) @ 0.25%	27.67	25.22	26.45 (9.91)	27.22	42.02	34.62 (14.34)
T_7 -Hydroxyl amine hydrochloride @ 0.25%	26.67	21.89	24.28 (0.90)	24.02	34.60	29.31 (-3.19)
Mean	27.09 (5.71)	25.63		27.02 (-32.93)	40.28	
Comparison of	S.Em±	CD (P=0.05)		S.Em±	CD (P=0.05)	
Variety (V)	0.19	0.54		0.18	0.52	
Treatment (T)	0.35	1.01		0.33	0.97	
V × T	0.49	1.43		0.47	1.37	

* - Figures in parenthesis indicate percentage change over control

5. DISCUSSION

A field experiment was conducted during the *rabi* season of 2011-12 to know the role of organic acids in amelioration of iron chlorosis of two groundnut varieties (var. GPBD-4 and TMV-2) grown in calcareous Vertisols and to know the iron reduction mechanisms in the plant, to assess iron concentration and examine the changes in the solution iron and DTPA-Fe compared to initial status are discussed in this chapter under the following headings.

5.1 Changes in different parameters before and after spraying of organic acids and at harvest of groundnut crop

5.1.1 Plant analysis before spraying

Leaf samples of two varieties from each treatment were analyzed for Fe^{2+}, Fe^{3+}, total iron, chlorophyll concentration and also pH of cell sap. In addition observations were made regarding visual chlorotic rating.

5.1.1.1 Ferrous (Fe^{2+}) iron concentration

Ferrous (Fe^{2+}) iron concentration was significantly influenced by two varieties of groundnut and was non-significant between the treatments and variety interaction (Fig. 2).

GPBD-4 variety recorded higher ferrous (Fe^{2+}) iron concentration of 19.64 per cent than TMV-2 variety. This may be due to fact that GPBD-4 variety is identified as Fe-efficient plant in comparison with TMV-2 as indicated by Nagarathnamma, (2011).

5.1.1.2 Ferric (Fe^{3+}) iron concentration

Ferric (Fe^{3+}) iron concentration was significantly influenced by the two variety of groundnut and was non-significant between the treatments and variety treatment interaction (Fig. 2).

GPBD-4 variety was recorded 1.8 per cent less ferric iron concentration when compared to TMV-2 variety. It was generally known that under iron stress conditions plants have the tendency to accumulate more of Fe^{3+} and it was not observed in case of GPBD-4 which is a Fe-efficient plant.

5.1.1.3 Total iron concentration

The total iron concentration did not differ significantly with treatments and varieties (Fig. 2). The Fe^{3+} and Fe^{2+} content inside the plant did not vary significantly among the varieties and also the treatments did not have significant effect obviously the observations were made prior to imposition of the treatments.

5.1.1.4 Total chlorophyll concentration

The chlorophyll concentration differed significantly with respect to variety and did not differ significantly with respect to treatments and variety-treatment interaction (Fig. 3).

GPBD-4 recorded 25.71 per cent higher chlorophyll concentration compared to TMV-2 variety. Iron is indirectly influences chlorophyll content in plants. Higher Fe^{2+} in GPBD-4 may be responsible for higher chlorophyll content in the variety than the other variety TMV-2.

5.1.1.5 pH of the cell sap

pH of the cell sap differ significantly with respect to varieties and did not differ significantly with respect to treatments and their interaction (Fig. 4).

The reduction in pH of cell sap is one of the mechanisms of utilizing Fe^{3+} by reduction of the ion. In the present study there was change in pH of the cell sap, thus mechanism of utilization of Fe^{3+} is related to pH change.

5.1.1.6 Visual chlorosis rating (VCR)

Visual chlorosis rating (VCR) was significantly influenced by two varieties of groundnut crop, but the treatments, variety and treatment interaction did not differ significant (Fig. 5).

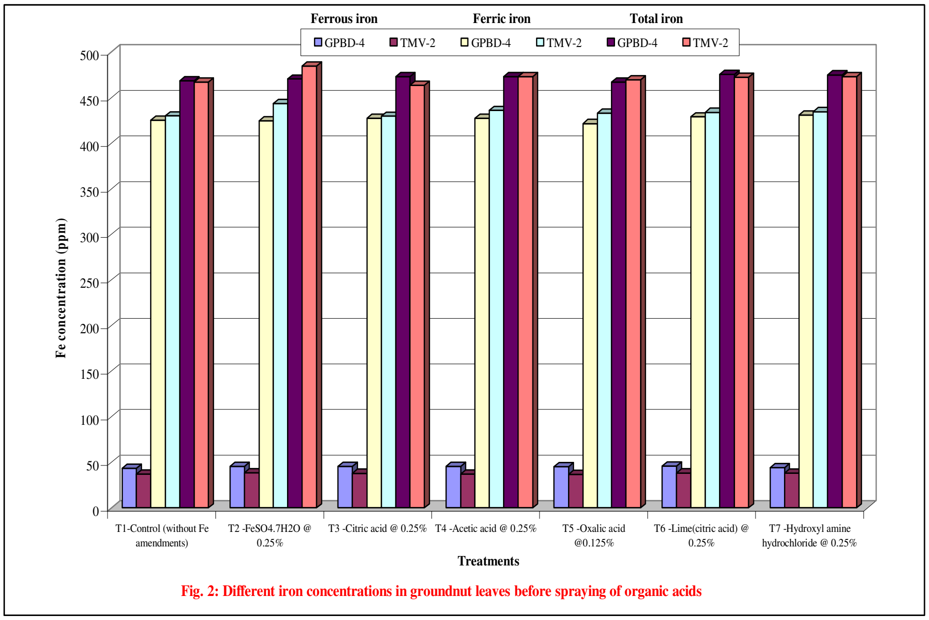

Fig. 2: Different iron concentrations in groundnut leaves before spraying of organic acids

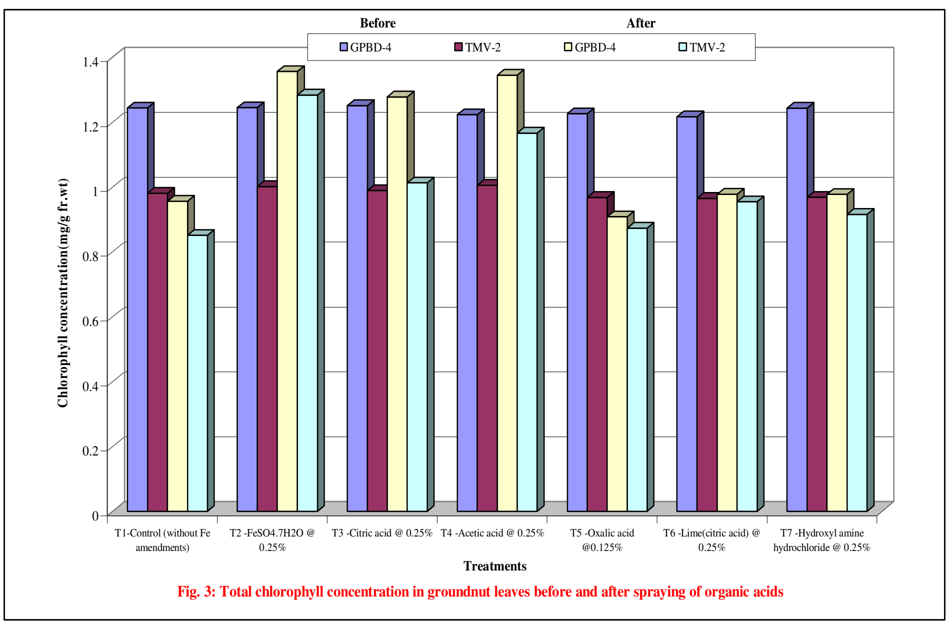

Fig. 3: Total chlorophyll concentration in groundnut leaves before and after spraying of organic acids

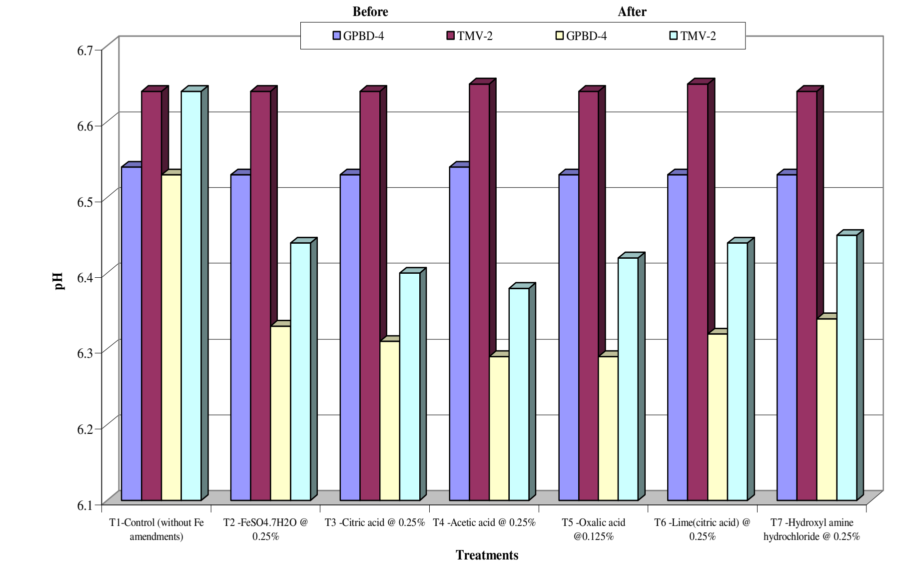

Fig. 4: pH of cell sap of groundnut leaves before and after spraying of organic acids

Fig. 4: pH of cell sap of groundnut leaves before and after spraying of organic acids

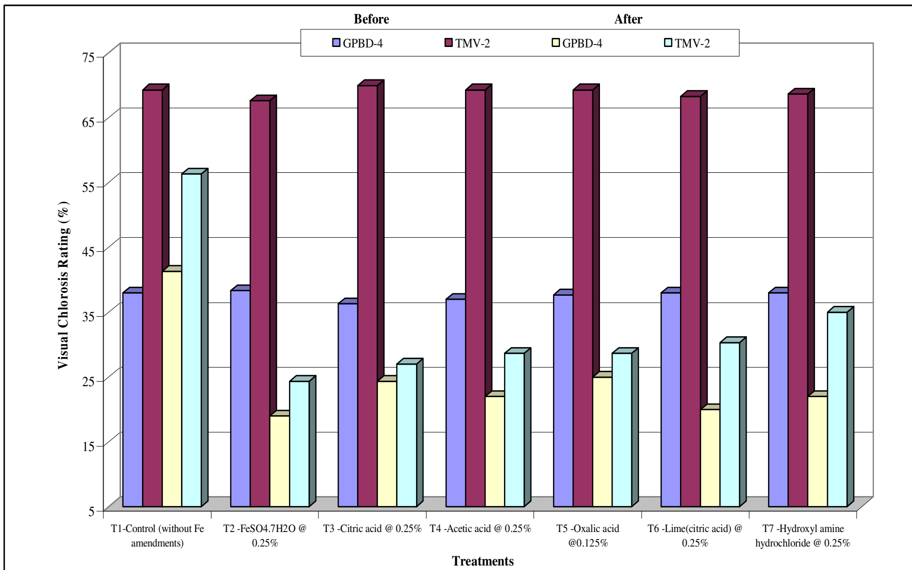

Fig. 5: Visual Chlorotic Ratings of groundnut leaves before and after spraying of organic acids

Fig. 5: Visual Chlorotic Ratings of groundnut leaves before and after spraying of organic acids

Visual chlorisis rating of GPBD-4 was 45.44 per cent less compared to TMV-2 variety. Higher chlorophyll content in GPBD-4 as indicated in 5.11.4 is reflected in VCR values. It can be said that there was significant lower VCR values for GPBD-4 compared to TMV-2 which was related to higher chlorophyll content in the variety.

5.1.2 Plant analysis after spraying

After spraying of organic acids, the same parameters which were observed before spraying was also recorded.

5.1.2.1 Ferrous (Fe^{2+}) iron concentration

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different variety, treatments and also in variety-treatment interactions (Fig. 6).

GPBD-4 recorded higher ferrous iron concentration of 1.90 per cent compared to TMV-2. Among the treatments, T_2 which received $FeSO_4.7H_2O$ spray, T_3 and T_6 which received citric acid either through chemical or natural source recorded higher ferrous iron concentration of 9.47 per cent 7.24 per cent and 5.29 per cent respectively when compared to control. This may be due to direct utilization of $FeSO_4.7H_2O$ and may be due to reduction of Fe^{3+} after spraying of organic acids. These results are in accordance with Olsen and Brown (1980), where the mobilization of iron was achieved by either pH reduction or a drop in redox potential or both, depending upon plant species and variety.

5.1.2.2 Ferric (Fe^{3+}) iron concentration

Ferric iron concentration was significantly influenced by different variety, treatments and their interactions (Fig. 6).

GPBD-4 variety recorded ferric iron concentration of 7.59 per cent less compared to TMV-2 variety. Among the treatments, treatment receiving lime (citric acid) @0.25% had higher ferric iron concentration of 24.65 per cent compared to control. However the treatments receiving $FeSO_4.7H_2O$ @ 0.25%, acetic acid @ 0.25% and hydroxyl amine hydrochloride @ 0.25% has shown significant increase of 4.30%, 4.30% and 11.63% respectively. This may be due to Fe^{3+} reduction to Fe^{2+} at different rates after spraying of different organic acids.

5.1.2.3 Total Fe concentration

Total iron concentration after spraying was significantly influenced by different treatments, varieties and their interaction (Fig. 6).

GPBD-4 variety recorded total iron concentration of 6.61 per cent less than TMV-2 variety. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher total iron concentration of 22.53 per cent compared to control.

5.1.2.4 Total chlorophyll concentration

Chlorophyll concentrations differed significantly between the variety, treatments and their interaction (Fig. 3).

Chlorophyll concentration in GPBD-4 was recorded 10.54 per cent more compared to TMV-2 variety. Among the treatments, treatment received $FeSO_4.7H_2O$ @ 0.25% was recorded higher chlorophyll concentration of 46.02 per cent compared to control. This may be due to higher the Fe^{2+} content and also lower pH value which resulted in higher chlorophyll in accordance with Mengel (1994).

5.1.2.5 pH of the cell sap

pH of the cell sap differed significantly between the varieties, treatments and their interaction (Fig. 4).

GPBD-4 was recorded pH value of 1.64 per cent less compared to TMV-2 variety. Among the treatments, treatment receiving acetic acid @ 0.25% was recorded 3.70 per cent less compared to control. This may be due to acid spray leads to reduce in pH which favors the increase in chlorophyll concentration. These results are in accordance Mengel (1994), where decrease in pH of the leaf apoplast noticed with the acid spray.

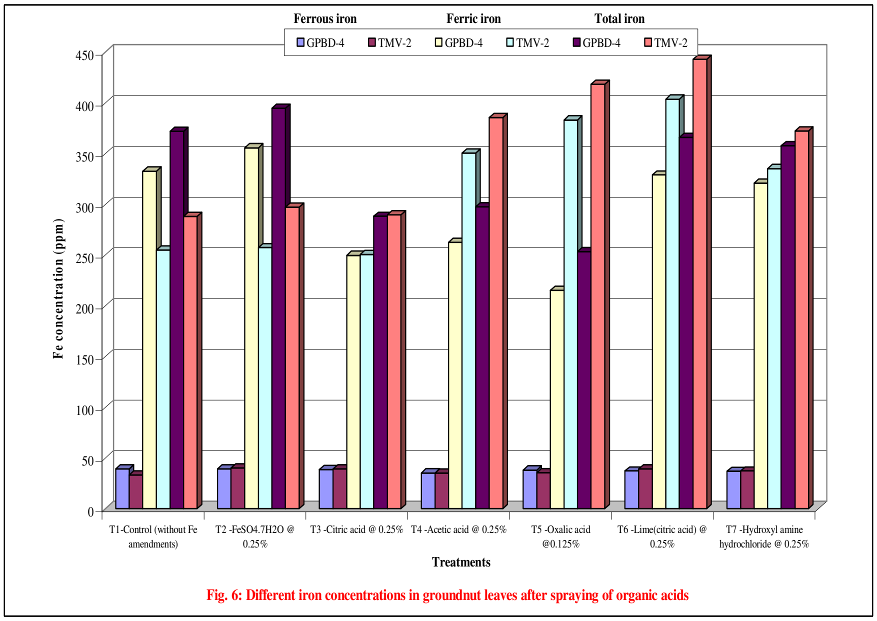

Fig. 6: Different iron concentrations in groundnut leaves after spraying of organic acids

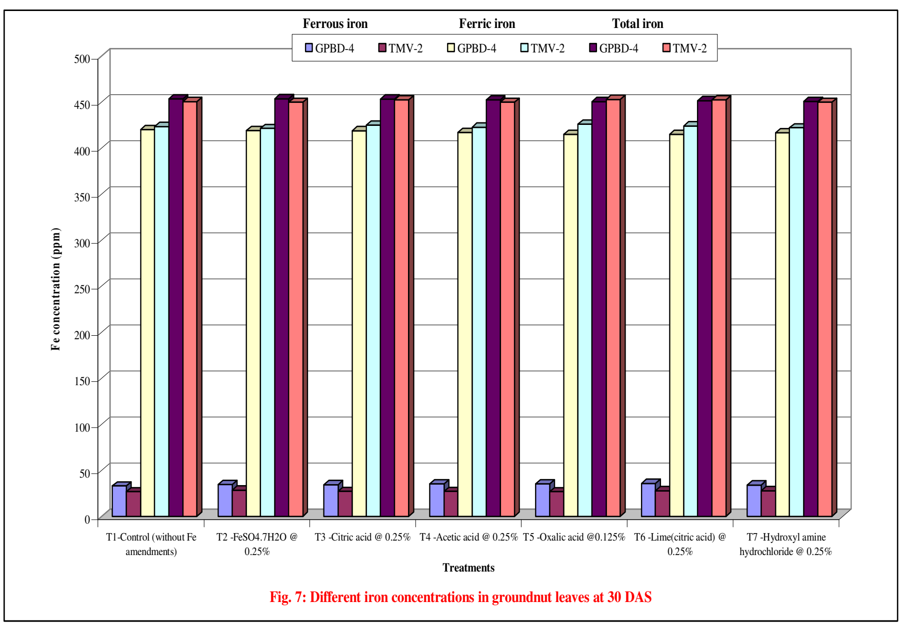

Fig. 7: Different iron concentrations in groundnut leaves at 30 DAS

5.1.2.6 Visual chlorosis rating (VCR)

Visual chlorosis rating (VCR) was significantly influenced by variety, treatments and their interaction (Fig. 5).

Visual chlorosis rating of GPBD-4 was 24.59 per cent less compared to TMV-2 variety. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% was 55.63 per cent less VCR compared to control. Higher the chlorophyll content obviously lower the visual chlorosis rating, due to increase in chlorophyll content after spraying in treatment which received $FeSO_4.7H_2O$ @ 0.25% recorded less VCR. However the treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @ 0.25% has shown significant decrease of 47.44, 48.12 and 48.46 per cent, respectively.

5.1.3 Plant analysis at 30, 60 DAS and at harvest

5.1.3.1 Ferrous (Fe^{2+}) iron concentration at 30 DAS

Ferrous (Fe^{2+}) iron concentration was significantly influenced by two varieties of groundnut and was non-significant with respect to treatments and variety interaction (Fig. 7).

GPBD-4 variety recorded higher ferrous (Fe^{2+}) iron concentration of 25.86 per cent than TMV-2 variety. This may be due to fact that GPBD-4 variety is identified as Fe-efficient plant in comparison with TMV-2 as indicated by Nagarathnamma, (2011).

5.1.3.2 Ferric (Fe^{2+}) iron concentration at 30 DAS

Ferric (Fe^{2+}) iron concentration was significantly influenced by the two varieties of groundnut and was non-significant between the treatments and variety treatment interaction (Fig. 7).

GPBD-4 variety was recorded 1.44 per cent less ferric iron concentration than TMV-2 variety. It is generally known that under iron stress conditions plants have the tendency to accumulate more of Fe^{3+} and it is not observed in case of GPBD-4 which has been reported as Fe-efficient plant (Nagarathnamma, 2011).

5.1.3.3 Total iron concentration at 30 DAS

The total iron concentration did not differ significantly with treatments and varieties (Fig. 7). The Fe^{3+} and Fe^{2+} content inside the plant did not vary significantly among the varieties and also the treatments did not have significant effect obviously the observations are made prior to imposition of the treatments.

5.1.3.4 Ferrous (Fe^{2+}) iron concentration at 60 DAS

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different variety, treatments and also in variety-treatment interactions (Fig. 8).

GPBD-4 recorded higher ferrous iron concentration of 20.21 per cent compared to TMV-2. Among the treatments, all the treatments was shown significant increase in ferrous iron concentration may be due to direct utilization of $FeSO_4.7H_2O$ and may be due to reduction of Fe^{3+} after spraying of organic acids.

5.1.3.5 Ferric (Fe^{3+}) iron concentration at 60 DAS

Ferric iron concentration was significantly influenced by different variety, treatments and their interactions (Fig. 8).

GPBD-4 variety was recorded ferric iron concentration of 11.10 per cent less compared to TMV-2 variety. Among the treatments, treatment receiving citric acid @ 0.25% had ferric iron concentration of 18.86 per cent less compared to control. However the treatments receiving oxalic acid @ 0.25% has shown 3.21 per cent less compared to control. This may be due to Fe^{3+} reduction to Fe^{2+} at different rates after spraying of different organic acids.

5.1.3.6 Total Fe concentration at 60 DAS

Total iron concentration after spraying was significantly influenced by different treatments, varieties and their interaction (Fig. 8).

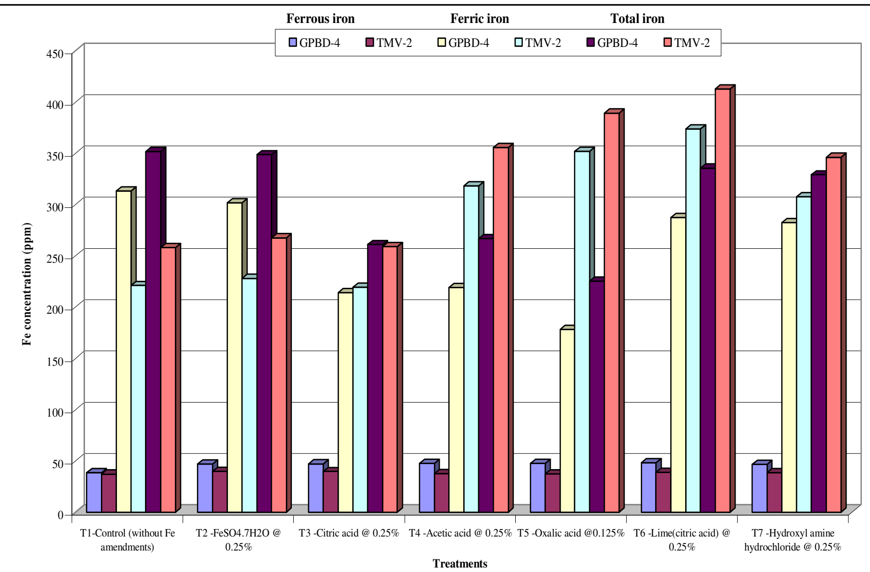

Fig. 8: Different iron concentrations in groundnut leaves at 60 DAS

Fig. 8: Different iron concentrations in groundnut leaves at 60 DAS

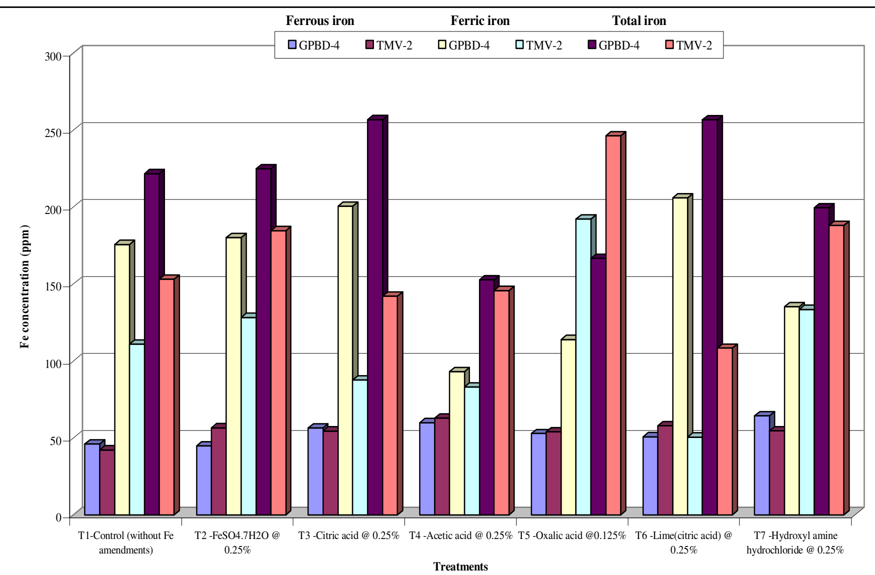

Fig. 9: Different iron concentrations in groundnut leaves at harvest

Fig. 9: Different iron concentrations in groundnut leaves at harvest

GPBD-4 variety was recorded total iron concentration of 7.45 per cent less than TMV-2 variety. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher total iron concentration of 22.67 per cent more compared to control.

5.1.3.7 Ferrous (Fe^{2+}) iron concentration at harvest

Ferrous (Fe^{2+}) iron concentration was significantly influenced by different varieties, treatments and also by variety and treatment interaction (Fig. 9).

At harvest GPBD-4 has recorded Fe^{2+} iron concentration of 1.98 per cent less than variety TMV-2. Among the treatments, treatment receiving acetic acid @ 0.25% had higher ferrous iron concentration of 38.89 per cent more compared to control. This may be due to fact that GPBD-4 is efficient in utilizing Fe^{2+} which leads to more utilization of Fe^{2+} hence lower the Fe^{2+} iron, whereas TMV-2 is inefficient in utilizing iron hence may be more Fe^{2+} at harvest. The treatment receiving acetic acid @ 0.25% had recorded high ferrous iron concentration where pH after spraying reduced.

5.1.3.8 Ferric (Fe^{3+}) iron concentration at harvest

Ferric iron concentration was significantly influenced by different varieties, treatments and their interaction (Fig. 9).

GPBD-4 has recorded higher ferric iron concentration of 40.52 per cent compared to TMV-2 variety. Among the treatments, treatment received $FeSO_4.7H_2O$ @ 0.25% was recorded higher value of 7.57 per cent more compared to control. In GPBD-4 more the utilization of ferrous iron more will be the ferric iron compared to TMV-2. The treatment received $FeSO_4.7H_2O$ @ 0.25% utilizes most of ferrous iron and leaving back more ferric iron.

5.1.3.9 Total Fe concentration at harvest

Total iron concentration at harvest was significantly influenced by variety, treatment and their interaction (Fig. 9).

Total iron concentration in GPBD-4 was 26.61 per cent more compared to TMV-2 variety. Among the treatments, treatment $FeSO_4.7H_2O$ @ 0.25% and lime (citric acid) @ 0.25 recorded higher total Fe value of 9.23 and 10.14 per cent respectively compared to control, because GPBD-4 is efficient in iron absorption in calcareous soils. The higher total Fe concentration was noticed in treatment receiving $FeSO_4.7H_2O$ @ 0.25% foliar spray. These results were in accordance with findings of Patel *et al.* (1993).

5.2 Soil analysis at harvest

5.2.1 DTPA iron

DTPA iron concentrations at harvest differed significantly with varieties, treatments and their interaction (Fig. 10).

DTPA iron was less in GPBD-4 variety, which was 6.12 per cent less compared to variety TMV-2. Among the treatments, treatment receiving oxalic acid @ 0.25% was recorded 38.42 per cent more DTPA iron concentration than control. The lower concentration of DTPA-Fe was recorded in GPBD-4 may be due to efficient utilization of DTPA-Fe compared to TMV-2.

5.2.2 Solution iron

Solution iron at harvest differed significantly among the variety, treatments and their interaction (Fig. 10).

GPBD-4 variety recorded 2.72 per cent less compared to TMV-2, among the treatments, treatment receiving citric acid @ 0.25% was recorded 26.86 per cent more compared to control.

GPBD-4 variety recorded less solution iron, this may be due to high utilization of solution iron by GPBD-4 compared to TMV-2. All the treatments recorded lower concentrations of solution iron (0.24 ppm) which was due to high $CaCO_3$ (19.5%) per cent. However the foliar spray of citric acid significantly increased solution iron. Here it is difficult to attribute the reason behind the increase in concentration of solution iron after spraying of citric acid.

5.2.3 Bicarbonate ion concentration

Bicarbonate ion concentration did not differ significantly among the treatments and varieties (Fig. 11). This may be due to that foliar spray did not make any influence on bicarbonate ion of a soil.

5.3 Growth parameters

5.3.1 Plant height

Plant height differed significantly with respect to varieties and did not differ significantly between the treatments at 30 DAS, whereas at 60 DAS, Plant height differed significantly with variety and treatments (Fig. 12).

GPBD-4 was recorded 9.94 per cent and 5.89 per cent more plant height than TMV-2 at 30 DAS and 60 DAS respectively. At 60 DAS plant height was 30.29 per cent more in treatment receiving $FeSO_4.7H_2O$ @ 0.25% compared to control. These results are in accordance with the results of Revathy *et al* (1997).

5.3.2 Number of pods per plant

Number of pods per plant significantly differed with treatment and varieties.

Number of pods in GPBD-4 was 6.08 per cent more compared to TMV-2 variety and among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% was recorded 38.91 per cent more than control. These results are in accordance with the results of Revathy *et al* (1997).

5.4 Yield parameters

5.4.1 Pod yield

Pod yield was significantly influenced by various treatments and two varieties (Fig. 13).

GPBD-4 variety was recorded 5.71 per cent more pod yield compared to TMV-2 variety. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% has shown 25.62 per cent more yield compared to control. Whereas, treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @ 0.25% have shown 18.48, 19.86 and 10.19 per cent more yield compared to control. The results are in accordance with Singh and Dayal (1992). All the treatments, except the treatment receiving hydroxyl amine hydrochloride have shown significant increase in pod yield. Organic acids were found to be significant in increasing pod yield over control. Tiffin (1967) reported that negatively charged Fe-containing compounds were essential for efficient iron movement through the xylem and citrate is the natural carrier of iron. These organic acids have an impact on many aspects of the physiology of iron deficient plants including excretion from roots and to supply the ferric chelate reductase enzymes with enough reducing power. This mechanism could be very important for plants growing in calcareous soils where an absolute Fe- deficiency does not takes place in presence of bicarbonate ion which is common in these soil conditions.

5.4.2 Haulm yield

Haulm yield differed significantly with variety and treatments and their interaction (Fig. 13).

Haulm yield in GPBD-4 recorded 32.93 per cent less when compared to TMV-2 variety, among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% had 26.38 per cent high haulm yield compared to control. The genetic character may influence the haulm yield.

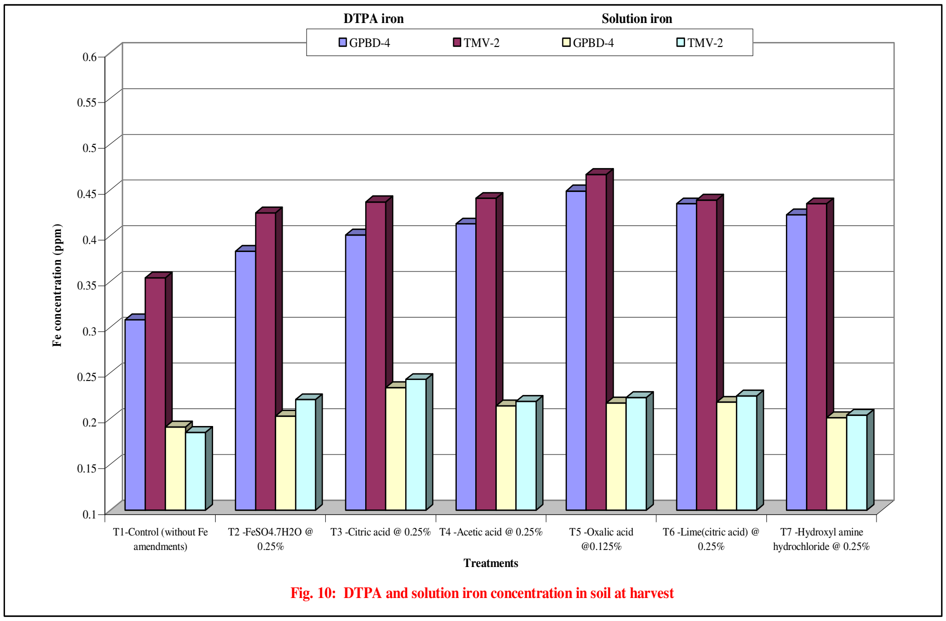

Fig. 10: DTPA and solution iron concentration in soil at harvest

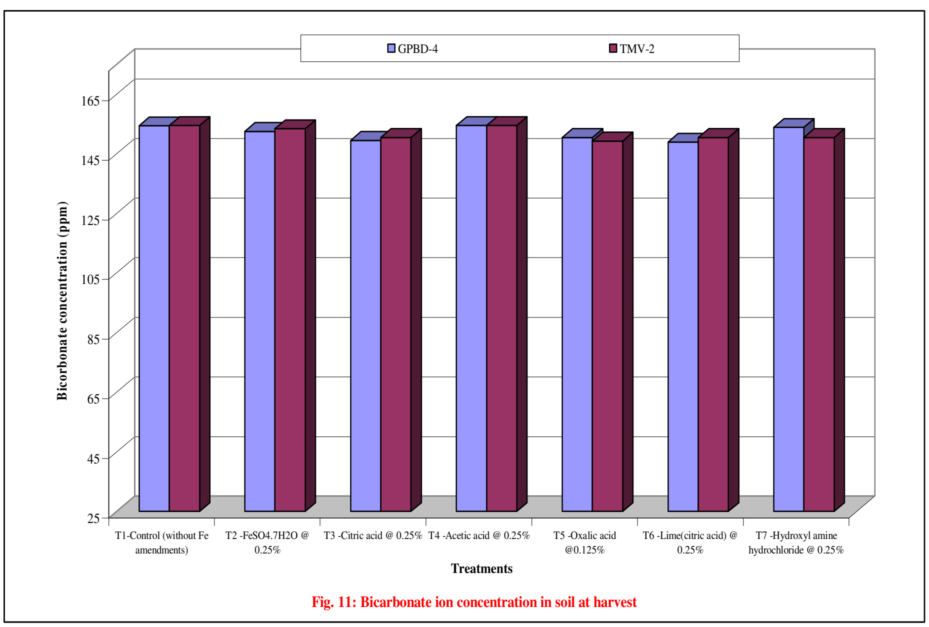

Fig. 11: Bicarbonate ion concentration in soil at harvest

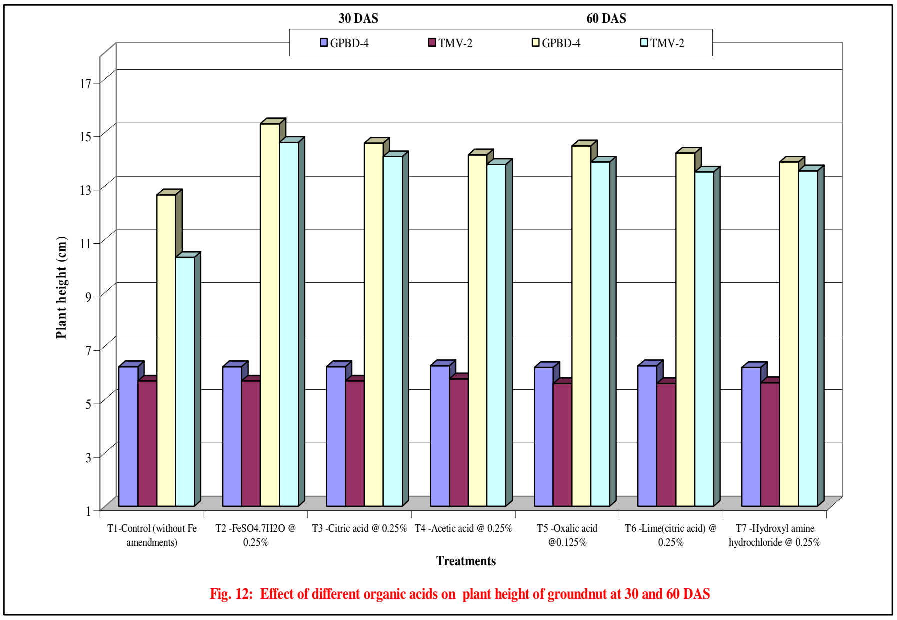

Fig. 12: Effect of different organic acids on plant height of groundnut at 30 and 60 DAS

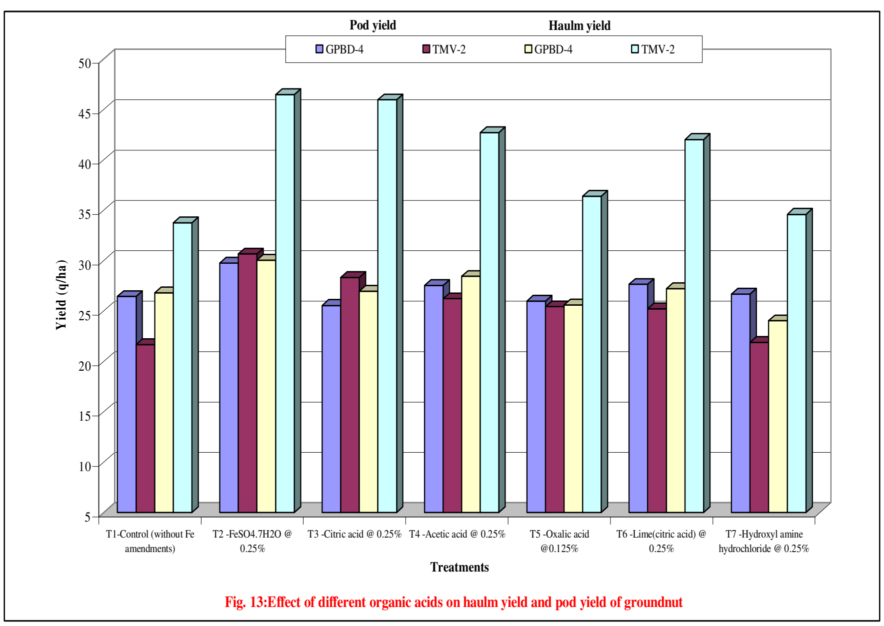

Fig. 13:Effect of different organic acids on haulm yield and pod yield of groundnut

6. SUMMARY AND CONCLUSIONS

A field experiment was conducted during the *rabi* season of 2011-12 to know the role of organic acids in amelioration of iron chlorosis in two groundnut varieties (var. GPBD-4 and TMV-2) grown in calcareous vertisols and also to assess different iron concentrations and to examine the changes in solution and DTPA-Fe in soil. Foliar application of $FeSO_4.7H_2O$ @ 0.25%, Citric acid @ 0.25%, Acetic acid @ 0.25%, Oxalic acid @ 0.125%, Lime (citric acid) @ 0.25% and Hydroxyl amine hydrochloride @ 0.25% were made.

Ferrous (Fe^{2+}) iron concentration was significantly influenced by two varieties of groundnut. GPBD-4 variety recorded higher ferrous (Fe^{2+}) iron concentration compared to TMV-2 variety. After spraying, GPBD-4 recorded higher ferrous iron concentration compared to TMV-2. Among the treatments, T_2 which received $FeSO_4.7H_2O$ spray recorded higher ferrous iron concentration compared to control. The increase in the iron concentration is due to direct utilization of $FeSO_4.7H_2O$ and may be the iron reduction due to decrease in pH of cell sap after spraying of organic acids.

After spraying, GPBD-4 variety recorded ferric iron concentration less compared to TMV-2 variety. Among the treatments, treatment receiving lime (citric acid) @0.25% had higher ferric iron concentration compared to control. However the treatments receiving $FeSO_4.7H_2O$ @ 0.25%, acetic acid @ 0.25% and hydroxyl amine hydrochloride @ 0.25% had recorded high ferric iron concentration. This may be due to Fe^{3+} reduction to Fe^{2+} at different rates after spraying of different organic acids. The treatment received $FeSO_4.7H_2O$ @ 0.25% utilizes most of ferrous iron and leaving ferric iron.

The Fe^{3+} and Fe^{2+} content inside the plant did not vary significantly among the varieties and also the treatments did not have significant effect before spraying. After spraying, GPBD-4 variety was recorded less total iron concentration than TMV-2 variety. Among the treatments, treatment receiving lime (citric acid) @ 0.25% had higher total iron concentration compared to control. At harvest, total iron concentration in GPBD-4 was more compared to TMV-2 variety. Among the treatments, treatment $FeSO_4.7H_2O$ @ 0.25% and lime (citric acid) @ 0.25% shown higher total Fe value compared to control, because GPBD-4 was efficient in iron absorption in calcareous soils. The higher total Fe concentration was noticed in treatment receiving $FeSO_4.7H_2O$ @ 0.25% foliar spray.

GPBD-4 recorded higher chlorophyll concentration compared to TMV-2 variety. Iron is indirectly influences chlorophyll content in plants. Higher Fe^{2+} in GPBD-4 may be responsible for higher chlorophyll content in the variety than the other variety TMV-2. After spraying, chlorophyll concentration in GPBD-4 was higher compared to TMV-2 variety. Among the treatments, treatment received $FeSO_4.7H_2O$ @ 0.25% had recorded higher chlorophyll concentration compared to control. This may be due to higher the Fe^{2+} content higher will be the chlorophyll and also lower the pH value higher the chlorophyll.

The reduction in pH of cell sap is one of the mechanisms of utilizing Fe^{3+} by reduction of the ion. In the present study there was change in pH of the cell sap, thus mechanism of utilization of Fe^{3+} is related to pH change. After spraying, GPBD-4 had recorded pH value less compared to TMV-2 variety. Among the treatments, treatment received acetic acid @ 0.25% had recorded less compared to control. This may be due to acid spray leads to reduction in pH which favors to increase in chlorophyll concentration.

VCR of GPBD-4 was less compared to TMV-2 variety. It can be said that there was significant lower VCR values for GPBD-4 compared to TMV-2 which is related to higher chlorophyll content in the variety. After spraying, VCR of GPBD-4 was less compared to TMV-2 variety. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% had shown less VCR compared to control. Higher the chlorophyll content obviously lower the VCR, due to increase in chlorophyll content after spraying in treatment received $FeSO_4.7H_2O$ @ 0.25% records less VCR. However the treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @0.25% has shown decrease in VCR.

DTPA iron was less in GPBD-4 variety compared to variety TMV-2. Among the treatments, treatment receiving oxalic acid @ 0.25% had recorded more DTPA iron concentration when compared to control.

aThe lower concentration of DTPA-Fe was recorded in GPBD-4 may be due to efficient utilization of DTPA-Fe compared to TMV-2. GPBD-4 variety recorded less solution iron compared to TMV-2, among the treatments, treatment receiving citric acid @ 0.25% had recorded more compared to control. GPBD-4 variety recorded less solution iron this may be due to high utilization of solution iron by GPBD-4 compared to TMV-2. All the treatments recorded lower concentrations of solution iron (0.24 ppm) which was due to high $CaCO_3$ (19.5%) percent. However the foliar spray of citric acid significantly increased solution iron. Bicarbonate ion concentration did not differ among the varieties and treatments this may be due to that foliar spray did not make any influence on bicarbonate ion of a soil.

GPBD-4 had recorded more plant height than TMV-2 at 30 DAS and 60 DAS. At 60 DAS plant height was more in treatment receiving $FeSO_4.7H_2O$ @ 0.25% compared to control. Number of pods in GPBD-4 was more compared to TMV-2 variety and among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% was recorded more number of pods when compared to the control.

Haulm yield in GPBD-4 was less when compared to TMV-2 variety, among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% had high haulm yield compared to control. The genetic character may influence the haulm yield.GPBD-4 variety had recorded more pod yield compared to TMV-2 variety. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% has shown more yield compared to control. All the treatments, except the treatment receiving hydroxyl amine hydrochloride had shown increase in pod yield. Organic acids were found to increase pod yield significantly over control. These organic acids have an impact on many aspects of the physiology of iron deficient plants including excretion from roots and to supply the ferric chelate reductase enzymes with enough reducing power. This mechanism could be very important for the plants growing in calcareous soils where an absolute Fe-deficiency does not take place even in the presence of bicarbonate content in the soil.

Future line of work

1. To study the mechanism of utilizing accumulated ferric iron concentration in the plant.
2. To study the rhizosphere for the uptake of nutrients a special reference to iron

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* - Originals are not seen

ROLE OF ORGANIC ACIDS IN AMELIORATION OF IRON CHLOROSIS IN GROUNDNUT (*Arachis hypogaea* L.) CROP GROWN IN A CALCAREOUS VERTISOL

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ABSTRACT

Iron is recognized as one of the main limiting factors of groundnut crop growth and yield in calcareous soils. To identify the effect of organic acids on concentration of Fe in the groundnut leaves, a field experiment was conducted in a calcareous soil at Amminbhavi village of Dharwad taluk during *rabi* 2011. Treatments included control (without Fe amendments), $FeSO_4.7H_2O$ @ 0.25%, Citric acid @ 0.25%, Acetic acid @ 0.25%, Oxalic acid @ 0.125%, Lime (citric acid) @ 0.25%, Hydroxyl amine hydrochloride @ 0.25% in a factorial randomized block design. Results of this study indicated that there was an increase in leaf Fe^{2+} content, chlorophyll content and decrease in visual clorotic rating (VCR) after spraying of $FeSO_4.7H_2O$ @ 0.25%. GPBD-4 recorded significantly higher ferrous iron concentration of 37.67 ppm compared to TMV-2 variety which recorded 36.97 ppm. Among the treatments, the treatment receiving $FeSO_4.7H_2O$ @ 0.25% was recorded higher ferrous iron concentration of 39.59 ppm. GPBD-4 recorded significantly higher chlorophyll concentration of 1.114 mg/g fresh weight compared to TMV-2 which recorded 1.008 mg/g fresh weight. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% recorded higher chlorophyll concentration of 1.320 mg/g fresh weight. However, the treatments receiving lime (citric acid) @ 0.25% and hydroxyl amine hydrochloride @ 0.25% were on par with one another. Among the varieties, GPBD-4 recorded higher pod yield of 27.09 q/ha as compared to 25.63 q/ha in TMV-2. Among the treatments, treatment receiving $FeSO_4.7H_2O$ @ 0.25% recorded higher pod yield of 30.22 q/ha. Whereas, treatments receiving citric acid @ 0.25%, acetic acid @ 0.25% and lime (citric acid) @ 0.25% were on par with one another, but significant over control. GPBD-4 variety with $FeSO_4.7H_2O$ @ 0.25% recorded highest yield over other treatment and variety treatment interaction.