

ORIGINAL ARTICLE

**Effect of iron dusts on physiological responses of gram seedlings
(*Cicer arietinum* L.) under laboratory conditions**

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A laboratory experiments was conducted for the assessment of physiological and biochemical responses of iron dust under the influence of different pH levels (6.5, 5.0, 3.0) and two concentration of iron dust (0.1 mg and 0.6 mg) with two particle size (100 μm and 300 μm) sprayed on the *Cicer arietinum* L. seed surface for fifteen day exposure. Observation was made on germination percentage and germination rate, vigour index, % phytotoxicity of root and shoot, chlorophyll, sugar, protein and proline content in both treated and control plant. The present results revealed that the seed color changes to brown under iron stress. The lower germination percentage and germination rate gradually decrease with pH of the medium but both the parameters were not significantly affected by the iron dust. Moreover higher % phytotoxicity was observed under all treatments compared to control and also lower values of this parameter were recorded in shoot than root. The reduction trend in chlorophyll and protein content was recorded at low pH but reverse result was recorded for sugar. Moreover highest proline was recorded under highly acidic condition.

Key words: Gram seeds (Cicer arietinum L.)/ pH level/ Iron dust/ Phytotoxicity/ Biochemical constituents

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The industries constitute a source of geographic and environmental disturbance due to mining and the emission of iron dust (Wong and Tam, 1977; Lopes *et al.*, 2000; Paling *et al.*, 2001). The iron dust, or iron ore particulate matter, represents the major pollutant released by these industries, during both the processing and storage of final products in open stock yards. These pollutants can be deposited either near the source or carried away, depending on the particle size, wind and landscape

features. This type of pollutant can directly affect photosynthesis, through abrasion, stomata blockage and smothering of the leaves, once the particles settle down on the organ surface (Hirano *et al.*, 1995; Naidoo and Chirkoot, 2004). Indirect effects may involve chemical and physical modification of the soil properties. Germination and the early growth stages are the most vulnerable periods of a plant life cycle; thus, any environmental stress, combined with the sensitivity

of the species, can interfere with a species establishment success (Fan and Wang, 2000; Grantz et al., 2003). This condition is likely to affect the vegetation dynamic, causing further ecological problems (Narayan et al., 1994; Wen et al., 2006). Iron hydroxides constitute the main ore exploited by the industries. Particulate matter derived from the crushing and beneficiation of iron ore is primarily inert and usually unavailable for plants as nutrient source. However, iron particulate accumulation in the soil due to heavy deposition or poor drainage, in combination with low pH of the substrate, may increase the availability of iron (Fe) to plants (Wong et al., 1978). Even though Fe is an essential micronutrient, high levels of this element in the soil can lead to toxicity or nutritional alterations, which can negatively affect plant metabolism (Connolly and Guerinot, 2002). The metal tolerance of plants may be attributed to different enzymes, stress proteins and phytochelatins (Van-Asche and Clijsters, 1990). The accumulation of metals at high concentration causes retardation of growth, biochemical activities and also generation of –SH group containing enzymes (Weckx and Clijsters, 1996). In the present investigation, Indian gram seed (*Cicer arietinum* L) is used to study the effect of different concentration of iron dust and pH of the medium on germination, vigour index, phytotoxicity, and changes of biochemical constituents under laboratory conditions.

MATERIALS AND METHODS

Gram seed (*Cicer arietinum* L.) were chosen to have their germination and initial growth tested under the influence of iron dust and different pH levels. The pH values studied were 6.5, 5.0 and 3.0, which were adjusted by adding diluted 0.1(N) nitric

acid and 0.1(N) NaOH solutions. The doses of iron dust were 0.1mg and 0.6 mg based on the average amount daily deposited in the vicinity of an iron ore industry (Lopes et al., 2000) and exposure of two particle size (100µm and 300µm). The pH without iron dust treatment was used as the control.

Healthy uniform gram seed (*C. arietinum*) was chosen as test plant and pre-soaked in distilled water for overnight. Before germination the gram seeds were surface sterilized with 0.1% HgCl₂ solution for 30 seconds and washing in double distilled water thoroughly for several times to remove excess of chemical and dried on absorbent to eliminate fungal attack. Sixteen selected seed were placed on moist sterile filter paper inside a sterilized Petri plate (9 cm dia. and 1.5 cm depth) for seed germination and seedling growth. The Petri dishes were covered with a net and kept in a growth room under optimum temperature (29 ± 1 °C) and adequate light ventilated condition. Light was supplied by four 40 watt white fluorescent lamps at a distance of 1 m (1,200 lux). Fifty seeds were placed in Petridish for germination. Observations were made for up to day 5 of incubation. Radical emergence of up to 1.5 mm was taken as a visible sign of germination. The length of shoot and root were recorded by using a centimeter scale, percentage of Phytotoxicity for shoot and root of 15 day old seedlings were calculated by the following formula (Chou and Lin, 1976).

$$\% \text{ Phytotoxicity} = \frac{\text{Root or shoot length of control} - \text{Root or shoot length of treatment}}{\text{Root or shoot length of control}} \times 100$$

The seedling vigour index (SVI) was calculated by the following method suggested by Abdul-Baki and Anderson (1993). SVI = germination percentage (shoot length + root length). For biochemical estimation of total chlorophyll (Arnon, 1949),

protein (Lowry *et al.*, 1951), soluble sugar (McCready *et al.*, 1950) and proline content (Bates, Waldren and Teare, 1973) were mediated from the leaves of the plants under various treatments of iron dusts using standard methods. Sample no. are S1 (pH 6.5), S2 (pH 5.0), S3 (pH 3.0), S4 (pH 6.5 and 0.1mg 100 μ m), S5 (pH 6.5 and 0.6mg 100 μ m), S6 (pH 5.0 and 0.1mg 100 μ m), S7 (pH 5.0 and 0.6mg 100 μ m), S8 (pH 3.0 and 0.1mg 100 μ m), S9 (pH 3.0 and 0.6mg 100 μ m), S10 (pH 6.5 and 0.1mg 300 μ m), S11 (pH 6.5 and 0.6mg 300 μ m), S12 (pH 5.0 and 0.1mg 300 μ m), S13 (pH 5.0 and 0.6mg 300 μ m), S14 (pH 3.0 and 0.1mg 300 μ m), S15 (pH 3.0 and 0.6mg 300 μ m).

RESULTS

The experimental results indicate that percentage of seed germination and germination rate was varying with pH of the medium (Fig. 1a, 1b). The lowest germination was recorded at lower pH (3.0), again in treatments S4 to S9 showed decrease in germination with pH but germination does not vary with amount of iron dust (100 μ m). This is clearly observed from Fig.(1a) where every set of treatment (S4 & S5, S6 & S7, S8 & S9) showed almost same germination pattern. Similar results were also recorded for treatment S10 to S15, where only variation is pH, without changing particle size (300 μ m) (Fig.1a). The vigour index of seedling in the all treatments decreased with increasing concentration of iron dust as well as particle size and also lower pH (Fig.2b). The percentage of phytotoxicity of root in all treatment showed an increasing trend with increasing concentration of iron dust than shoot. The highest % phytotoxicity of both root and shoot was noted in S14 and S15 and lowest in S4 (37.19% in root and 21.68% in shoot) 1.

compared to control (Fig. 2a). Again during the germination period most of the gram seeds under iron dust exposure developed a dark color on the seed coats. The results of the pigment content showed that chlorophyll 'a', 'b' and total chlorophyll gradually decreased with increasing acidity of the medium. Again keeping pH constant (6.5), with increasing exposure of iron dust 0.1mg to 0.6 mg chlorophyll 'a', 'b' and total chlorophyll drastically reduced but again increased with decreasing the pH of the medium. However, more intense reduction of chlorophyll 'a', 'b' and total chlorophyll was recorded for further increasing the acidity of the medium. The iron dust which was added in treatment S4 to S9 was 100 μ m but when size of the iron again increased to 300 μ m, it was found that pattern of pigment reduction is same as before (Fig.3a). Sugar content in root for all the treatments are very low compared to shoot and leaves except S10 where sugar content in shoot much less than root and leaves. It was found that highest sugar content was recorded at pH 3.0 and lowest at pH 6.5 in shoot and leaf respectively. But application of iron dust (100 μ m and 300 μ m) doses not provides any significant sugar content in different parts of the plant (Fig. 3c). Protein content was recorded highest in root (S1, S4, S5 and S9) and lowest in shoot (S1, S4, S10, and S13). The variation of protein content was also noted pH dependent. With decreasing the pH of the medium from 6.5 to 3.0, the protein content reduces from 337.06 mg/g to 2.18 mg/g. Similarly shoot protein content reduces from 89.03mg/g to 0.98 mg/g with increasing the acidity of the medium (Fig. 3b). Again unchanged results in protein content was recorded when particle size of iron dust vary with pH. Interestingly it was found that there was no variation of secondary metabolites when amount of

iron dust changes with constant pH. Again the results of proline content was recorded different in different pH of the medium. Similar unchanged

results of proline was recorded when pH of the medium kept constant with variation of iron dust in different sizes (Fig.3d).

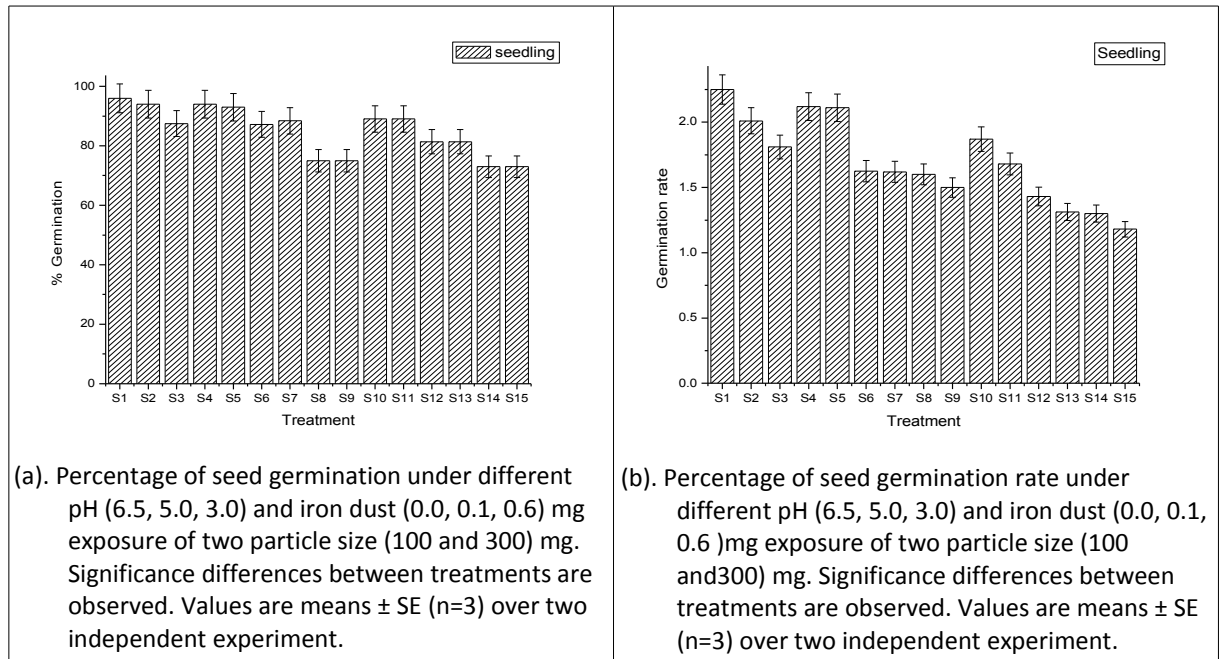


Figure 1. Percentage of seed germination and germination rate of gram seedling under different treatments after 15 day of sowing of iron dust.

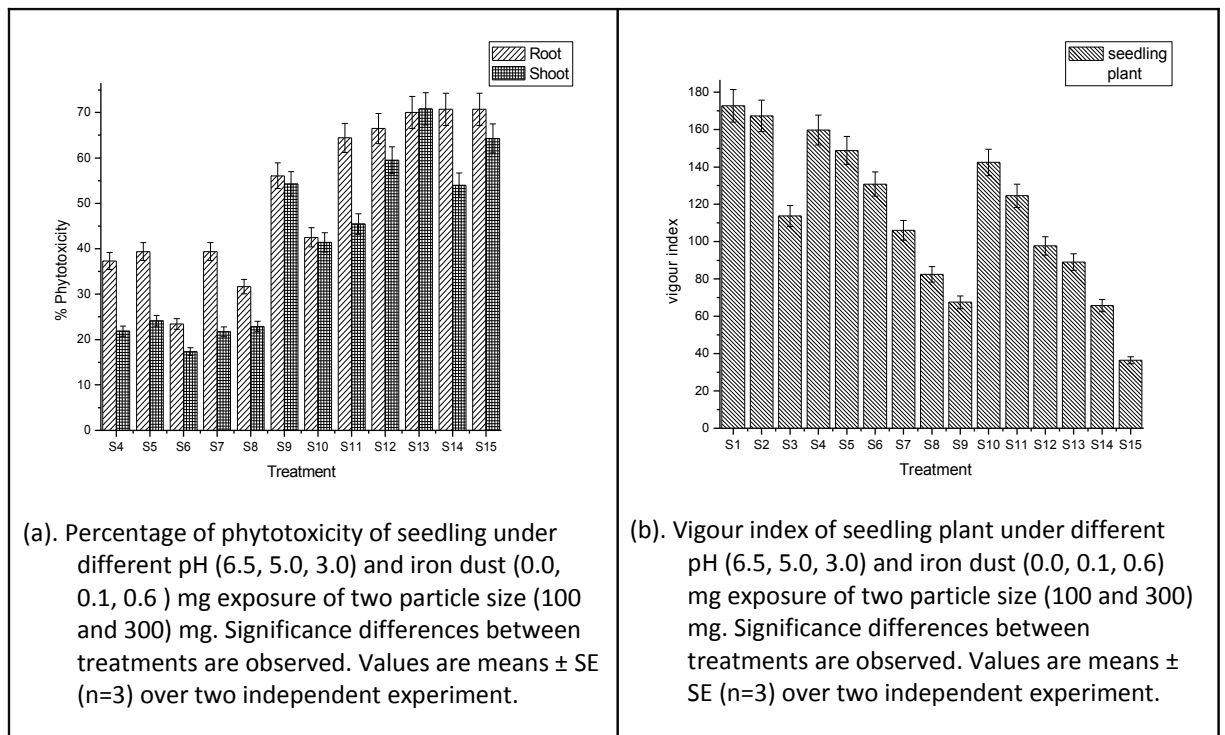


Figure 2. Percentage of phytotoxicity and vigour index of gram seedling under different treatments after 15 day of sowing of iron dust.

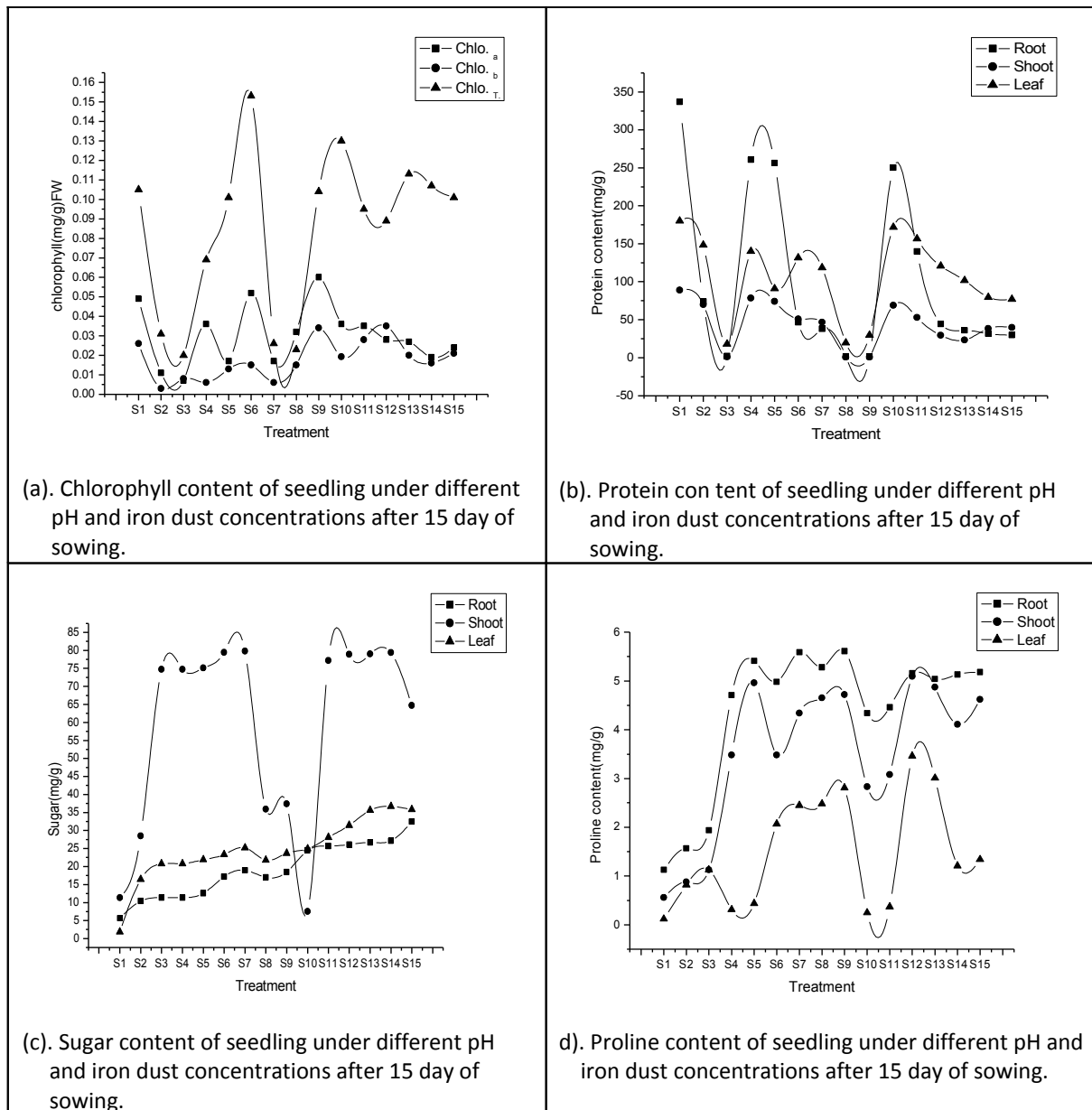


Figure 3. Biochemical parameters of seedling under different pH and iron dust exposure after 15 day of sowing.

DISCUSSION

Both percentage of germination and rate of germination of seeds was highly affected by pH of the medium. This is quite possible because seed physiology greatly influenced by the acidity of the medium (Fan and Wang, 2000). Moreover, highly acidic condition disrupts the whole metabolic processes including glycolysis which probably due to imbalance of pH gradient within the cells structure (Kuki *et al.*, 2009). The vigour index

reduces under acidic condition is due to changes the nature of permeability of seed coat and subsequently its affects on germination and which is indicates the overall performance of the seeds and seedlings (Abirami *et al.*, 2010). Percentage of Phytotoxicity of root was found to be more than shoot, this is due to the fact that metal accumulation on root due to binding of metal on the cell wall of root and retarded cell division and cell elongation (Woolhouse, 1983). From the

experimental findings it was revealed that, the color change in seed coat during germination is usually related to phenol oxidation (Rashid *et al.*, 2005). Phenols are commonly present in many parts of the seeds, including the coat and embryo, and they are primarily related to the regulation of seed germination as well as to defense against herbivores and pathogen infestation (Muscolo *et al.*, 2004; Rashid *et al.*, 2005). In contrast, the browning of the gram seed coat in the treatments with iron dust was probably due to phenol oxidation by the metal. The presence of elemental Fe⁰, as in iron dust, can cause oxidation of phenol to quinines (Rush *et al.*, 1995). Hence, it is thought that the oxidizing characteristic of the iron dust might have accelerated the oxidation of seed coat phenols and/or contributed to the accumulation of internal Fe in the seeds of *Cicer arietinum* which might have affected the germination process. Chlorophyll content showed reduction trend with decreasing pH of the medium is probably due to the damage of chloroplast under such acidic condition. Singh and Srivastava, (2002) endorsed the same for incorporation of cement kiln dust into leaf tissues. Similar trend of reduction was reported by Pandey *et al.*, (1999) in maize crop, Pandey and Simba, (1990b) for water melon, and Pandey and Simba, (1989) for gram leave. Soluble sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and breakdown during respiration. The concentration of soluble sugar is indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution. The sugar content was found to be reduced with the increase in the of amount iron dust applied. Reduction in soluble sugar content under stress condition can be attributed to

increased respiration and decreased CO₂ fixation because of chlorophyll deterioration (Tripathi and Gautam, 2007). The same observation was reported by Uma and Ramana Rao (1996) where plants polluted by dust. The reduction in protein content might be due to the results of decreased photosynthesis and/or break down of existing protein or due to reduced *de novo* synthesis (Singh and Jothi, 1999). In the present study revealed that the protein content was found to be decreased in all treatment sets. This reduction in protein content might be due to the enhanced rate of protein denaturation (Tripathi and Gautham, 2007; Prasad and Inamdar, 1990a). The enhanced protein denaturation and breakdown of existing protein to amino acid is the main cause of reduction in protein content (Constantinidou and Kozlowski, 1979).). In our study, we found that prolin concentration is significantly higher when iron exposure was increased. This accumulation of prolin has also been reported as being a sign of stress in plant (Rai *et al.*, 2003) which at higher concentration act as a solute for intercellular osmotic adjustment (Silveira *et al.*, 2003).

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