Effect of mode and source of iron nano particles on the biological properties of the calcareous soil

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Abstract
The effect of mode and source of iron application on biological properties was evaluated in the greenhouse experiment along with the different sources i.e. nano Fe$_{2}$O$_{3}$ and FeSO$_{4}$ in the calcareous soil with wheat as test crop during kharif season of 2014, New Delhi. Application of FeSO$_{4}$ through both soil and foliar modes significantly increased the dehydrogenase activity by 53 and 38%; whereas application of iron nano particles at 0.3 mg Kg$^{-1}$ though soil enhanced the activity by 26% over the control. Foliar application of 0.2% mg Fe either through FeSO$_{4}$ and Fe$_{2}$O$_{3}$ (nano) doses exhibited the similar response of towards the microbial biomass carbon during the entire crop growth stages. Salts of FeSO$_{4}$ and nano Fe$_{2}$O$_{3}$ did not influence both the bacterial and fungal population in the calcareous soil. Overall results revealed that the application of lower doses of nano Fe$_{2}$O$_{3}$ through foliar and soil showed non-significant and negative impact on soil biological properties.

Keywords: Mode and iron nano, biological, calcareous soil

Introduction
In India, wheat is grown in 30.7 million ha, the production level is 98.9 million tones and the productivity is about 3368 kg/ha (Ministry of Agriculture and Farmers Welfare, GOI). Wheat is one of the most important food crops and feeds more than 40 per cent population of India. The area under wheat crop was increased dramatically by 3.2 times; production has increased over 15 times in 2016-17, respectively, over the base year of 1950-51. Increased production has surged up the usage of fertilizers in the package of practices to meet the nutrient demand of rice crop. Fertilization has been extensively used as a common management practice to maintain soil fertility, soil health and crop productivity (Shen et al., 2010) [9]. Application of chemical fertilizers are extensively followed across the crop production practices to ensure the nutrient availability to the plant growth. Hence, application of fertilizers could modify the soil biological conditions because of availability of abundant nutrient for microbial proliferation. Nanotechnology encompasses a range of technologies related to the manipulation of matter at the length scale of 1–100 nm. Generally, nano scale materials possess transitional behaviour between molecule and their bulk materials which can show multiple changes in chemical, physical properties. Introduction of nanomaterials has mounted and exploited up in different fields of science i.e. energy, environment and health sciences. However, use of nanotechnology in agriculture is in toddler stage and growing in slower way. A series of work on nano particles on plant growth, nutrient accumulation and bio diversity has been carried out by researchers. Microorganisms are active participator in bio geochemical cycle namely carbon, nitrogen, sulphur and phosphorus in soil (Sadowsky and Schortomezeyer, 1997) [7]. Use of nano particle such as carbon nanotubes and fullerenes do not show any impact on the soil microbial community (Tong et al., 2007) [10]. On the contrary, Ge et al., (2011) [3] reported that soil bacteria were adversely affected by applied nano ZnO and TiO$_{2}$. In Indian context, intrusion of nano technology in field crops is still not documented clearly and not understood well. With this review, impact of iron nano particles on soil biological properties were studied and presented here.

Materials and Methods
Greenhouse experiment was conducted to assess the impact of synthesised nano Fe$_{2}$O$_{3}$ in calcareous soil using wheat (Triticum aestivum var HD – 2958) as test crop.
For the experiment, Surface soil (0-15 cm) samples were collected from a Research Farm of Rajendra Agricultural University, Pusa, Samastipur, Bihar. The collected soil samples belong to Typic calcicorthents located in the hot sub humid agro climatic zone (annual precipitation 1107 mm) of Indo Gangetic Plain (25°98’ N, 85°67’ E; 52 m above mean sea level). The soil samples were air-dried, ground using wooden mortar and pestle and sieved through 2 mm sieve. The resulted Fe2O3 nano particles were formulated to see the effect on soil biological properties with the following treatments: T1-Control (recommended dose of fertilizers); T2 -of 0.2% Fe (Foliar; FeSO4); T3- 0.2% Fe (Foliar; nano Fe2O3); T4- 0.04% Fe (Foliar; nano Fe2O3); T5- 15 mg Fe kg⁻¹ (Soil; FeSO4); T6- 3 mg Fe kg⁻¹ (Soil; nano Fe2O3); T7- 0.6 mg Fe kg⁻¹ (Soil; nano Fe2O3). Destructive soil samples were collected at different intervals i.e. 30 DAT, 60 DAT and Maturity, respectively. Dehydrogenase activity (DHA) was estimated by monitoring the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC) as suggested by Klein et al. (1971). Results of dehydrogenase activity was expressed as µg TPF formed per gram soil in 24 hours on oven dry weight basis. Microbial biomass carbon (MBC) in harvested soil was estimated the method provided by Jenkinson and Powlson (1976). Moist soils (10 g each) were taken in a three different sets of beaker. One set of beaker was kept for fumigation with chloroform for 24 hours; another set of beakers was kept unfumigated. Third set with moist soil was used for estimation of moisture content in the soil. After reaction, samples (fumigated and non-fumigated) were digested with 0.5 M potassium sulphate (K₂SO₄) at 120 °C for 2 hours in a digestion block with the vial was kept in digestion tube to capture the evolved carbon dioxide from the digestion process which was trapped in vial containing 0.1 N sodium hydroxide (NaOH). Simultaneously, control was also running with 0.5 M potassium sulphate in place of the soil sample extract. The excess or unreacted NaOH was titrated using standard 0.01 N sulphuric acid (H₂SO₄) with phenolphthalein indicator. The MBC values were calculated using the formula given below,

\[
\text{MBC (mg kg}^{-1}\text{)} = \left( C_{f} - C_{d} \right) / K_{EC}
\]

Where, \( C_{f} \) = carbon in fumigated soil, \( C_{d} \) = carbon in unfumigated soil, \( K_{EC} \) = efficiency of extraction (0.25).

**Enumeration of Bacterial and Fungi**

Microbial count of bacteria and fungi was measured following method of Waksman et al., (1922). The dilution plate technique with nutrient agar and rose Bengal was used to culture the bacteria and fungi, respectively. Inoculated plates were further incubated at 28 ± 2 °C and incubation period varies with bacteria (2 days) and fungi (3-4 days).

**Results and Discussion**

**Dehydrogenase Activity**

Soil application of 15 mg Fe kg⁻¹ (FeSO₄), 3 mg Fe kg⁻¹ Fe₂O₃ (nano) and 0.6 mg Fe kg⁻¹ Fe₂O₃ (nano) registered soil DHA values as 4.96, 4.09 and 3.46 mg TPF g⁻¹ h⁻¹, respectively under wheat (Table 1). Foliar application of 0.2% Fe (FeSO₄), 0.2% Fe (Fe₂O₃ nano) and 0.04% Fe (Fe₂O₃) registered the DHA values under wheat were 4.46, 3.64 and 3.64 mg TPF g⁻¹ h⁻¹. Dehydrogenase activities of wheat were 5.46, 3.62 and 2.75 mg TPF g⁻¹ h⁻¹, at 30 DAS, 60 DAS and maturity, respectively. Interactive effects of applied Fe and growth stages on dehydrogenase activity in soil were significant under both the crops. From such results, it is difficult to make out the reason, which is responsible of imparting positive effect of nanoparticle on root exudates. In case of soluble sources of Fe i.e. sulphate salt as used in the present study might have positively influenced normal metabolic activity, growth and development of crops, which were grown on Fe deficient calcareous soil. Such positive effect on overall condition of plant health might have resulted into secretion of root exudates. On the other hand, in case of nanoparticles, entry of such insoluble particle into plant body enter through root or foliage might have created stress condition which was resulted into excretion of significantly higher amount of root exudates over control (Huang et al., 2014) [4]. Cullen et al., (2011) [8] reported that soil application of nano zero valent iron at 10 mg kg⁻¹ increased the dehydrogenase activity in loam soil. Sarvendra Kumar (2012) [8] reported an inhibitory effect on dehydrogenase activity, when a higher dose of ZnO and fullereen nano particles were applied. In case of growth stages, dehydrogenase activity was decreased over the period of time in both the crops with Fe nutrition attributed to the fact that release of root exudates has normally been reduced after flowering (Huang et al., 2014) [4].

**Microbial biomass carbon**

Soil under wheat shown significant response in terms of microbial biomass carbon to the added FeSO₄ and Fe₂O₃ (nano) (Table 1). The microbial biomass carbon was significantly higher as 221 mg kg⁻¹ due to foliar application of 0.2% Fe solution through Fe₂O₃ (nano) over control. Whereas, application of 15 mg Fe kg⁻¹ (FeSO₄), 3 mg Fe kg⁻¹ (Fe₂O₃ nano) and 0.6 mg kg⁻¹ (Fe₂O₃ nano) registered the MBC values as 193, 174 and 181 mg kg⁻¹, respectively, which were statistically at pat with the control. On an average, increase in age of the plants was associated with the reduction in microbial biomass carbon values being 223, 190 and 170 mg kg⁻¹, at 30 DAS, 60 DAS and maturity, respectively under wheat. Interactive effect of growth stages and iron sources on soil microbial biomass carbon was statistically significant under wheat. In earlier section, it is observed that application of nanoparticle either to soil or foliage resulted into increase in dehydrogenase activity, which was inferred to be due to excretion of root exudates by plant roots under stress condition. Such positive effect of applied nano Fe₂O₃ on MBC may be explained based on the fact that enhanced excretion of root exudates might have helped in proliferation of microorganisms. Peralta-Videa et al., (2016) and Huang et al., (2014) [4] reported that application of nano Cu increased the secretion of root exudates in Cucumber plants. Advancement of growth stages in crops significantly altered the microbial biomass carbon and the highest MBC was observed in 30 DAS (223 mg kg⁻¹) in wheat. Microbial biomass carbon is generally influenced by growth stages of plant and organic matter content in soil (Yang et al., 2010) [11].

**Bacterial and fungal population**

The effect of different rates and sources of iron (Fe) application on bacterial and fungal population (log CFU) in soil at different growth stages of wheat is presented in Figure 1 & 2. Experimental data on application of sources and modes of iron on bacterial and fungal population found to be non-significant under wheat soil. However, increasing of age of plants altered the bacterial population in soils under wheat with values as 6.74, 6.80 and 6.76 logCFU, at 30 DAT, 60 DAT and maturity, respectively. Soil application of 15 mg Fe
kg\(^{-1}\) (FeSO\(_4\)), 3 mg Fe kg\(^{-1}\) (nano Fe\(_2\)O\(_3\)) and 0.6 mg Fe kg\(^{-1}\) (nano Fe\(_2\)O\(_3\)) were registered the same population (4.16 logCFU) under wheat. However, growth stages of wheat had significant impact on fungal population under wheat and values were 4.09, 4.23 and 4.11 logCFU at 30 DAS, 60 DAS and maturity, respectively. Interactive effect of different growth stages and sources on bacterial and fungal population in soil was not significant. Soil microbes would be expected to respond differently to fertilizer treatments as plant activity varies through growth stages (Yoshida, 1981). Exposure of soil to different sources of Zn and Fe (sulphate salt and nanoparticles) did not cause any impact on the bacterial and fungal population in soil under rice. In case of wheat, significant enhancement in fungal population was recorded in soil at 2.5 mg Zn kg\(^{-1}\) (ZnSO\(_4\)) as 4.25 logCFU which was at par with that 0.5 mg Zn kg\(^{-1}\), where only 1/5\(^{th}\) of Zn was applied through nano ZnO. It may be ascribed to nano particle induced root exudation in soil, which might have helped in proliferation of microorganisms. Pallavi et al., (2016) \(^{[5]}\) reported the altered organic acid compositions and releasing patterns of root exudates in cowpea and Brassica, when treated with Ag nano particles. In present study, plant growth stages played the most critical role in explaining the variation in the population of microbes. Interestingly, bacterial and fungal population remain unchanged in soil over the different growth stages under wheat.

**Table 1:** Effect of rates, sources and modes of iron application on dehydrogenase activity (TPF \(\mu\)g g\(^{-1}\) hr\(^{-1}\)) and microbial biomass carbon (mg kg\(^{-1}\)) in soil at different growth stages of wheat

| Treatments                        | Dehydrogenase activity | Microbial Biomass carbon |
|-----------------------------------|-------------------------|--------------------------|
|                                   | 30 DAS | 60 DAS | Maturity | Mean | 30 DAS | 60 DAS | Maturity | Mean |
| Control                           | 3.55   | 3.30   | 2.84     | 3.23  | 193    | 197    | 185      | 192  |
| Foliar                            |         |         |          |       |        |        |          |      |
| 0.2% Fe (FeSO\(_4\))             | 5.83   | 4.13   | 3.39     | 4.46  | 223    | 212    | 190      | 208  |
| 0.2% Fe (nano Fe\(_2\)O\(_3\))   | 5.01   | 3.35   | 2.55     | 3.64  | 262    | 194    | 209      | 221  |
| 0.04% Fe (nano Fe\(_2\)O\(_3\))  | 5.70   | 3.07   | 2.16     | 3.64  | 188    | 196    | 191      | 191  |
| Soil                              |         |         |          |       |        |        |          |      |
| 15 mg Fe kg\(^{-1}\) (FeSO\(_4\))| 7.93   | 3.65   | 3.30     | 4.96  | 241    | 209    | 129      | 193  |
| 3 mg Fe kg\(^{-1}\) (nano Fe\(_2\)O\(_3\)) | 6.19  | 3.95   | 2.14     | 4.09  | 207    | 158    | 158      | 174  |
| 0.6 mg Fe kg\(^{-1}\) (nano Fe\(_2\)O\(_3\)) | 4.01  | 3.91   | 2.87     | 3.46  | 249    | 164    | 131      | 181  |
| Mean                              | 5.46   | 3.62   | 2.75     |        | 223    | 190    | 170      |      |

LSD (p=0.05) = Least significant difference
DAT = Days after transplanting
DAS = Days after sowing

![Fig 1: Effect of iron sources on bacterial population (logCFU) at different stages of wheat](image)
Fig 2: Effect of iron sources on fungal population (logCFU) at different stages of wheat

Conclusion
Soil and foliar application of Fe through sulphate salt enhanced the dehydrogenase activity (DHA) in soil under wheat. Whereas meagre improvement was observed in nano particle treated soil and the activity was to tune of 26% over control. Foliar application of 0.2% mg Fe either through FeSO₄ and Fe₂O₃ (nano) doses were exhibited the similar response of towards the microbial biomass carbon during the entire crop growth stages. While microbial population in soil (bacterial and fungal) remained unaffected due to soil application of Fe and low effect was pronounced only in case of foliar application. Overall results revealed that the application of lower doses of nano Fe₂O₃ (0.3, 0.06 mg kg⁻¹ soil and 0.2 and 0.04% spray) through soil and foliar did not have any negative impact on the biological properties of calcareous soil. Thus to obtain a clear picture on the impact on the microbial population and enzyme activities can be tried with higher doses may be formulated and to be further evaluated.

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