International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 3334-3337

Received: 04-05-2020
Accepted: 06-06-2020

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

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DOI: https://doi.org/10.22271/chemi.2020.v8.i4ap.10166

Abstract
The effect of mode and source of iron application on biological properties was evaluated in the green house experiment along with the different sources *i.e.* nano Fe_2O_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_2O_3 (nano) doses were exhibited the similar response of towards the microbial biomass carbon during the entire crop growth stages. Salts of $FeSO_4$ and nano Fe_2O_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_2O_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 fertilisers 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 Schortomeyer, 1997) [7]. Use of nano particle such as carbon nanotubes and fullerene does 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_2O_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 calciorthents* 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 Fe_2O_3 nano particles were formulated to see the effect on soil biological properties with the following treatments; T_1-Control (recommended dose of fertilizers); T_2 - of 0.2% Fe (Foliar; $FeSO_4$); T_3- 0.2% Fe (Foliar; nano Fe_2O_3); T_4- 0.04% Fe (Foliar; nano Fe_2O_3); T_5- 15 mg Fe kg^{-1} (Soil; $FeSO_4$); T_6- 3 mg Fe kg^{-1} (Soil; nano Fe_2O_3); T_7- 0.6 mg Fe kg^{-1} (Soil; nano Fe_2O_3). 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 (K_2SO_4) at 120 ºC for 2 hours in a digestion block with the vial was kept in digestion tube to capture the evolved carbon di oxide 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 (H_2SO_4) with phenolphthalein indicator. The MBC values were calculated using the formula given below,

$$MBC\ (mg\ kg^{-1}) = (C_f - C_{uf})/ K_{EC}$$

Where, C_f = carbon in fumigated soil, C_{uf} = 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 ºC 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} ($FeSO_4$), 3 mg Fe kg^{-1} Fe_2O_3 (nano) and 0.6 mg Fe kg^{-1} Fe_2O_3 (nano) registered soil DHA values as 4.96, 4.09 and 3.46 µg TPF g^{-1} h^{-1}, respectively under wheat (Table 1). Foliar application of 0.2% Fe ($FeSO_4$), 0.2% Fe (Fe_2O_3 nano) and 0.04% Fe (Fe_2O_3) registered the DHA values under wheat were 4.46, 3.64 and 3.64 µg TPF g^{-1} h^{-1}. Dehydrogenase activities of wheat were 5.46, 3.62 and 2.75 µg 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) [2] 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 fullerene 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 $FeSO_4$ and Fe_2O_3 (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 Fe_2O_3 (nano) over control. Whereas, application of 15 mg Fe kg^{-1} ($FeSO_4$), 3 mg Fe kg^{-1} (Fe_2O_3 nano) and 0.6 mg kg^{-1} (Fe_2O_3 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 Fe_2O_3 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 (logCFU) 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_2O_3) and 0.6 mg Fe kg^{-1} (nano Fe_2O_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 µg 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_2O_3)	5.01	3.35	2.55	3.64	262	194	209	221
0.04% Fe (nano Fe_2O_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_2O_3)	6.19	3.95	2.14	4.09	207	158	158	174
0.6 mg Fe kg^{-1} (nano Fe_2O_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)	Source (S)=0.44, Day (D)=0.29, SxD =0.75				Source (S)= 29, Day (D)=19, SxD=50			

LSD = Least significant difference
DAT = Days after transplanting
DAS = Days after sowing

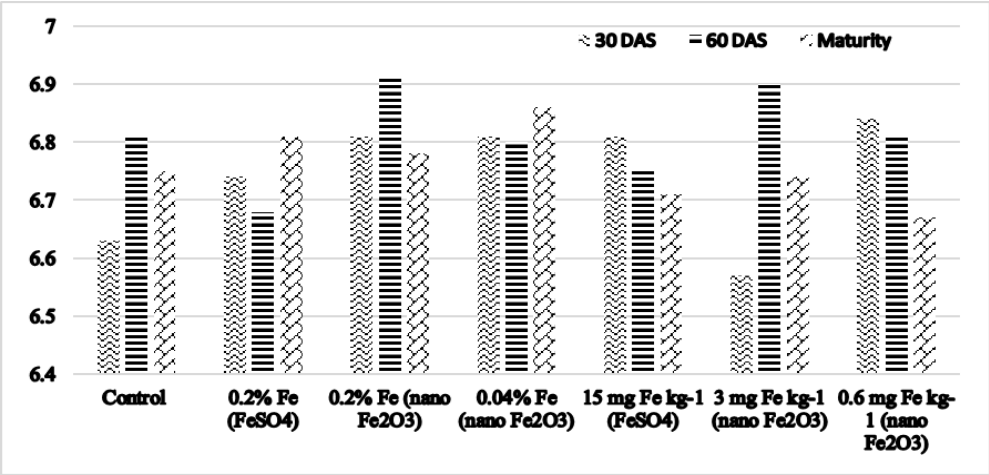

Fig 1: Effect of iron sources on bacterial population (logCFU) at different stages of wheat

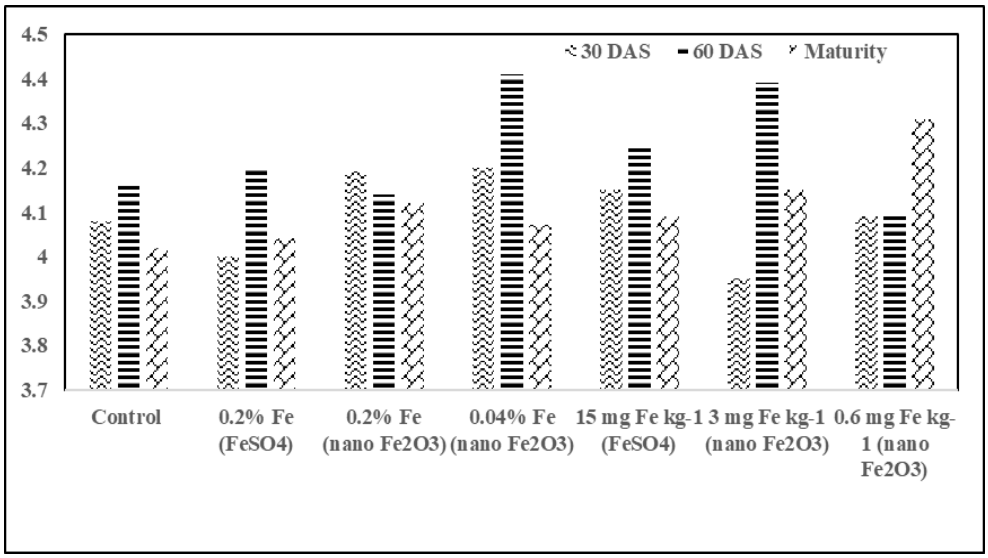

Fig 2: Effect of iron sources on fungal population (logCFU) at different stages of wheat

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 $FeSO_4$ and Fe_2O_3 (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 Fe_2O_3 (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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