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EFFECT OF NITRATE AND MANGANESE APPLICATION ON MANGANESE POOLS IN SOIL AND ITS UPTAKE IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Ammonium- fed plants may acidify the rhizosphere and thus increase the availability of Mn in calcareous alkaline soils. So, in order to evaluate the effect of nitrate and manganese application on Mn pools in soil and its uptake in wheat, a pot experiment was conducted in the screen house of Department of Soil Science, CCS HAU, Hisar. Result showed that a significant increased in yields, Mn content and its uptake in wheat and Mn pools in soil with the application of NO_3^- and Mn. The highest yields of grain and straw were recorded with 90 mg NO_3^- kg^{-1} and 25 mg Mn kg^{-1} soil application. In wheat plant, maximum Mn content and its uptake were recorded with highest application of 90 mg NO_3^- kg^{-1} and 50 mg Mn kg^{-1}soil. Among various Mn fractions, the highest Mn concentration was found associated with residual (RES) form followed by MnOX > AFeOX > CFeOX > EX > CARB > OM. These results showed that nitrate and Mn application is a good predictor of Mn availability in Mn deficient light texture soils and to sustain productivity and quality of wheat on such soils, the judecious use of Mn in proper amount becomes essential.

KEY WORDS
Fraction
Manganese
Nitrate, Uptake, Wheat, Grain and Straw Yield

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INTRODUCTION

Green revolution has helped in increasing the food production, thereby greatly reduced starvation, calories and protein malnutrition. The shrinking of agricultural land and the demand for more food production calls for multiple cropping in a single piece of land (Singh *et al.,* 2014). However, this caused greater depletion of micronutrient reserve in soil and thereby accentuated wide spread deficiency of these nutrients. Their deficiencies are causing not only hidden hunger but also leading to entire failure of crops and lower content of trace elements in plant parts. Manganese (Mn) is one of the main essential micro-nutrients which have an important contribution in crop production. In the recent past its deficiency has become a widespread nutritional disorder of crops in many countries of the world, which often results in decreased crop yields (Sadana *et al.,* 2002).

Plants quantitative and qualitative yield is strongly dependent on plant nutrition. In most regions of the world, use of chemical fertilizers is very imbalanced and is not based on plant requirement. The soils of Haryana are known to be getting depleted in available micronutrients due to continuous cropping, high yielding varieties, intensive cropping system, increased use of fertilizers and irrigation facilities without externally supplementing these nutrients (Fageria *et al.,* 2002). Wheat is an important cereal crop of Haryana State. Extension of rice cultivation in coarse textured and alkaline soils of Haryana also leads to widespread deficiency of micronutrient, particularly, Mn in wheat crop which is emerging up year by year. During submergence, solubility of Mn increases appreciably because of its reduction and continuous leaching of Mn from upper to lower soil layers during rice cultivation results in its deficiency in the succeeding winter season crops especially wheat (Gangoi, 1984). No doubt, manganese requirements of plants are generally low, but with the increasing use of high analysis micronutrient free fertilizers, Mn deficiency is likely to be intensified particularly in light texture soils due to leaching losses and is going to be a major constraint in realising yield potential of high yielding varieties of wheat (Nayyar *et al.,* 1990).

Manganese occurs in various forms in soils together with smaller amount associated with organic matter. Understanding the mechanism of Mn distribution in different fraction helps to know its retention in soils and release to plants (Shuman, 1979). Variations in soil properties, however, play a major role in influencing the distribution of Mn among various chemical pools. Due to its cationic nature and solubility of its common salt, leaching losses of Mn are generally high. Therefore, the movement of Mn in the soil determines the magnitude of its losses in drainage water and soil profile. Such information is essentially required for making sound fertility programmes. The hypothesis has been addressed in the present study to evaluate the effect of nitrate (NO_3^-) and manganese (Mn) application on yields, Mn content and its uptake in wheat grain and straw and Mn pools in soil.

MATERIALS AND METHODS

Pot experiment on wheat was performed using four kg each thoroughly mixed soil (0-15 cm) depth collected from Village Dhani Lamba, District Fatehabad, a Mn deficient- area in the state was filled in plastic pots. The experiment comprising of the four level of NO_3^- (0, 30, 60, 90 mg kg^{-1}) applied through ammonium nitrate and three level of Mn (0, 25, 50 mg kg^{-1}) as manganese sulphate was laid out in factorial completely randomized design (CRD) in the screen house according to Steel and Torrie (1980) and all the treatment replicated thrice. Recommended doses of N, P and K as basal was done using 50 mg N kg^{-1} soil as urea, 50 mg P kg^{-1} soil as potassium di-hydrogen phosphate (KH_2PO_4) and 15 mg K kg^{-1} soil as potassium sulphate (K_2SO_4) were applied in each pot. Ten viable seeds of wheat variety (WH-1105) were sown on 21th November, 2014 in each pot. Thinning was done after fifteen days and four uniform healthy plants per pot were allowed to grow up to maturity. Pots were irrigated with distilled water as and when required.

The crop was harvested after 142 days of its sowing. Grain and straw samples were collected and washed in 0.1N HCl solution and thereafter in deionised water to remove soil and any other foreign materials. The grain and straw samples were first air dried and finally dried in an oven at 65 ± 2^0C till a constant weight was obtained. Thereafter, grain and straw yield was recorded and samples of grain and straw were ground in a stainless steel grinder and digested with di-acid mixture of HNO_3 and $HClO_4$ (4:1). The Mn content in plant digests was estimated with the help of Atomic Absorption Spectrophotometer (Varian-Spectra AA-240 FS). After crop harvesting, soil samples were collected from each pot, dried under room temperature and ground with a wooden pestle and mortar. The samples were sieved through a 2 mm sieve and stored in polythene bags for analysis of different fractions of Mn in soil.

pH and EC were determined in (1:2) soil : water suspension with the help of glass electrode pH meter and conductivity meter bridge, respectively as per the methods described by Richard (1954). Organic Carbon (OC) was estimated by wet digestion method (Walkley and Black, 1934). Calcium Carbonate ($CaCO_3$) was estimated by rapid titration method (Puri's, 1930). Mechanical analysis was done using international pipette method (Piper, 1966). Available-Mn content in soil samples was determined by DTPA methods of Lindsay and Norvell (1978) using Atomic Absorption Spectrophotometer (Varian-Spectra AA-240 FS). Total - Mn content in soil fractions were extracted by hydrofluoric-perchloric acid mixture as prescribed by Page *et al.,* (1982) and estimated on Atomic Absorption Spectrophotometer (Varian-Spectra AA-240 FS).

A seven step sequential fractionation procedure of Tessier *et al.,* (1979) was followed for Mn partition into exchangeable (EX), carbonates bound (CARB), organic matter bound (OM), Mn oxide bound (MnOX), amorphous Fe oxide bound (AFeOX), crystalline Fe oxide bound (CFeOX) and residual fraction (RES) is given in Figure 2. Manganese in all extracts was determined by Atomic Absorption Spectrophotometer (Varian-Spectra AA-240 FS).

RESULTS AND DISCUSSION

Physico-chemical characteristics of experimental soils

A representative soil sample before fertilization was air-dried and analysed for some relevant physic-chemical properties and DTPA-extractable Mn. Data presented in Table 1 revealed that the soil used in pot experiment was slightly alkaline in nature with soil pH of 8.10. The electrical conductivity of soil was 0.40 dS m^1. Organic carbon content and calcium carbonate were found 0.40% and 1.10 %. Texturally, soil had the composition: sand 82.50 percent, silt 13.30 percent and clay 4.2 percent and was classified as loamy sand. The

Table 1: Some relevant physico-chemical characteristics of experimental soil

Characteristic	Value
pH (1:2)	8.1
EC (dSm^{-1})	0.4
Organic carbon (%)	0.4
Calcium carbonate (%)	1.1
Sand (%)	82.5
Silt (%)	13.3
Clay (%)	4.2
Textural class	Loamy sand
DTPA-Mn (mg kg^{-1})	2.1

Table 2: Wheat yields (g pot^{-1}) as affected by NO_3^- and Mn application

NO_3^- level(mg kg^{-1})	Mn level (mg kg^{-1})			Mean
Grain yield	0	25	50	
0	6.22	8.00	7.57	7.26
30	6.42	8.50	7.76	7.56
60	6.49	9.57	7.84	7.96
90	6.57	9.96	8.10	8.21
Mean	6.42	9.00	7.82	
CD (p = 0.05)	Mn = 0.27,	NO_3^- = 0.31	Mn × NO_3^- = 0.55	
Straw yield				
0	11.29	11.21	10.02	10.84
30	11.36	13.03	10.63	11.67
60	11.46	13.60	13.50	12.85
90	11.50	14.08	13.64	13.07
Mean	11.40	12.98	11.94	
CD (p = 0.05)	Mn = 0.42,	NO_3^- = 0.48	Mn × NO_3^- = 0.84	

Table 3: Grain and straw Mn concentration (mg kg^{-1}) of wheat as affected by NO_3^- and Mn application

NO_3^- level(mg kg^{-1})	Mn level (mg kg^{-1})			Mean
Grain	0	25	50	
0	6.53	12.53	19.21	12.76
30	8.35	15.18	19.25	14.26
60	8.98	16.06	23.50	16.18
90	9.16	17.90	24.93	17.33
Mean	8.25	15.42	21.72	
CD (p = 0.05)	Mn = 2.22,	NO_3^- = 2.56	Mn × NO_3^- = NS	
Straw				
0	20.81	28.30	37.51	28.87
30	21.98	28.46	40.33	30.25
60	22.25	29.18	41.05	30.82
90	23.86	33.28	41.35	32.83
Mean	22.22	29.80	40.06	
CD (p = 0.05)	Mn = 5.39,	NO_3^- = NS	Mn × NO_3^- = NS	

Table 4: Grain and straw Mn uptake (μg pot^{-1}) of wheat as affected by NO_3^- and Mn application

NO_3^- level(mg kg^{-1})	Mn level (mg kg^{-1})			Mean
Gain	0	25	50	
0	40.57	100.16	145.41	95.38
30	53.80	129.09	149.38	110.75
60	58.15	154.85	184.24	132.41
90	60.23	179.38	201.93	147.18
Mean	53.18	140.87	170.24	
CD (p = 0.05)	Mn = 20.97,	NO_3^- = 24.21	Mn × NO_3^- = NS	
Straw				
0	235.08	316.13	375.85	309.02
30	248.01	372.72	428.70	349.81
60	255.19	383.12	554.17	397.49
90	274.78	450.10	582.20	435.69
Mean	253.26	380.51	485.23	
CD (p = 0.05)	Mn = 66.72,	NO_3^- = NS	Mn × NO_3^- = NS	

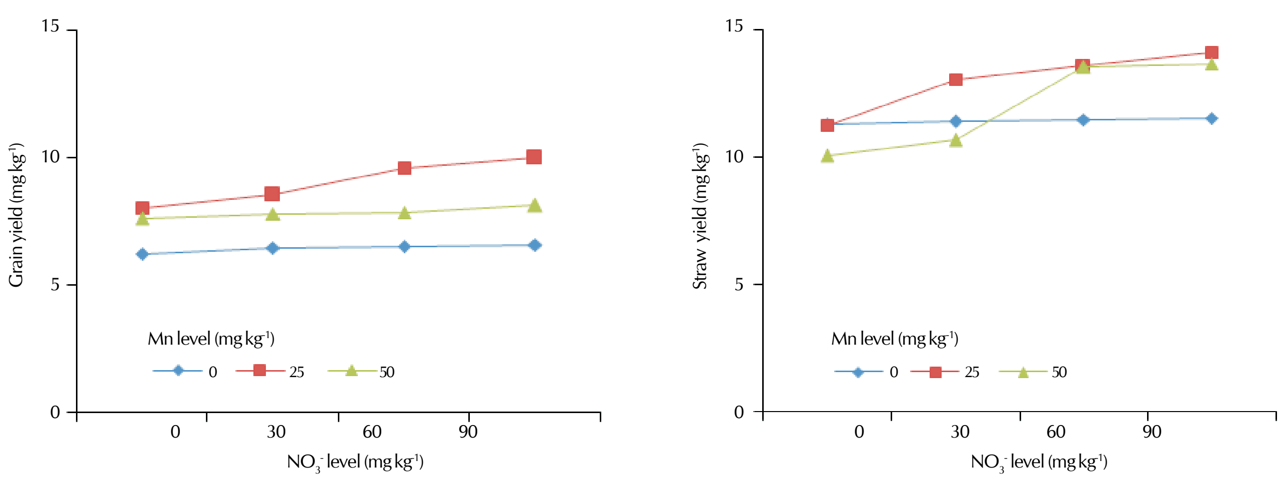

Figure 1: Interaction effect of NO_3^- and Mn application on wheat yield

native content of DTPA-Mn was found 2.10 mg kg^{-1}. The experimental soil was almost low in organic carbon content and DTPA-Mn content.

Grain and straw yield of wheat

The data on NO_3^- and Mn fed wheat revealed that grain and straw yields increased significantly with NO_3^- and Mn application (Table 2). Increases in grain yield over control at 30, 60 and 90 mg NO_3^- kg^{-1} levels were 4.13, 9.64 and 13.08 percent over control, respectively. The corresponding increases in straw yield were 7.65, 16.88 and 20.57 percent. The highest yields of grain and straw were recorded with 90 mg NO_3^- kg^{-1} addition. However, the yield of grain at 25 and 50 mg Mn kg^{-1} levels were 40.18 and 21.80 percent, while straw yield was recorded 11.66 and 5.70 percent over control. The highest grain and straw yields were recorded with 25 mg

Table 5: Effect of NO_3^- and Mn application on Mn pools (mg kg^{-1}) in soil

NO_3^- level(mg kg^{-1})	Mn level (mg kg^{-1}) 0	25	50	Mean
Exchangeable (EX) –Mn				
0	0.81	0.84	0.92	0.85
30	0.82	0.87	0.97	0.90
60	0.86	1.00	0.97	0.94
90	0.88	1.02	1.01	0.97
Mean	0.85	0.93	0.96	
CD (p = 0.05)	Mn = 0.03,	NO_3^- = 0.04	Mn × NO_3^- = 0.07	
Carbonate bound (CARB) – Mn				
0	0.19	0.20	0.20	0.19
30	0.22	0.21	0.26	0.23
60	0.26	0.25	0.26	0.25
90	0.26	0.27	0.27	0.26
Mean	0.23	0.24	0.25	
CD (p = 0.05)	Mn = NS,	NO_3^- = NS	Mn × NO_3^- = NS	
Organic matter bound (OM) – Mn				
0	0.01	0.02	0.03	0.02
30	0.01	0.03	0.03	0.02
60	0.04	0.04	0.04	0.04
90	0.08	0.04	0.06	0.06
Mean	0.03	0.03	0.04	
CD (p = 0.05)	Mn = NS,	NO_3^- = 0.01	Mn × NO_3^- = 0.02	
Mn oxide bound (MnOX) – Mn				
0	47.89	72.85	78.49	66.41
30	48.56	73.57	79.98	67.34
60	49.26	73.72	81.02	68.00
90	51.40	76.19	81.42	69.67
Mean	49.27	74.08	80.21	
CD (p = 0.05)	Mn = 1.74,	NO_3^- = 2.01	Mn × NO_3^- = NS	
Amorphous Fe oxide (AFeOX) – Mn				
0	12.13	34.16	39.33	28.54
30	13.81	37.20	39.81	30.26
60	14.53	37.77	40.14	30.81
90	14.55	38.25	40.27	31.02
Mean	13.75	36.84	39.87	
CD (p = 0.05)	Mn = 0.58,	NO_3^- = 0.67	Mn × NO_3^- = 1.17	
Crystalline Fe bound (CFeOX) – Mn				
0	9.57	11.44	11.82	10.94
30	9.70	12.18	12.31	11.40
60	10.47	13.46	13.47	12.47
90	11.76	14.49	14.45	13.56
Mean	10.38	12.89	13.01	
CD (p = 0.05)	Mn = 0.98,	NO_3^- = 1.13	Mn × NO_3^- = NS	
Residual (RES) – Mn				
0	198.15	160.56	149.96	169.56
30	206.21	156.10	153.22	171.86
60	204.45	159.29	165.72	176.49
90	206.52	165.44	175.72	183.89
Mean	203.84	160.36	162.15	
CD (p = 0.05)	Mn = 3.14,	NO_3^- = 3.63	Mn × NO_3^- = 6.29	
Total – Mn				
0	268.75	280.04	280.75	276.51
30	279.33	280.16	286.58	282.02
60	279.87	285.50	300.62	288.66
90	285.45	295.70	313.20	298.12
Mean	278.35	285.35	295.28	
CD (p = 0.05)	Mn = 2.02,	NO_3^- = 2.34	Mn × NO_3^- = 4.05	

Mn kg^{-1} soil application. From these results, it may be concluded that 90 mg NO_3^- kg^{-1} and 25 mg Mn kg^{-1} soil application is optimum dose for proper growth and development of wheat. The significant response of wheat to manganese in the present study may be accounted for the low amount of DTPA-Mn in soil. The interaction effect of NO_3^- and Mn on grain and straw yields was significant (Table 2 and Figure 1). This may be due to the stimulating effect of NO_3^- application which improves the physiological performance of plant. Similar finding were also reported by Abbas *et al*.

		Soil (10 g)
		↓
Exchangeable Mn	←------------------	40 ml 1 M Mg $(NO_3)_2$(Shake 2 hrs.)
		↓
		Soil
		↓
Carbonate bounded Mn	←------------------	40 ml 1 M NaOAc(pH 5.0, CH_3COOH) (Shake 5 hrs.)
		↓
		Soil
		↓
Organically bounded Mn	←------------------	20 ml 0.7 M NaOCl(pH 8.5) (30 min. in boiling water bath. Stir occasionally, Repeat extraction)
		↓
		Soil
		↓
Manganese oxide Mn	←------------------	50 ml 0.1 M $NH_2OH.HCl$(pH 2, HNO_3) (Shake 30 min.)
		↓
		Soil
		↓
Amorphous Fe oxides Mn	←------------------	50 ml 0.25 $MNH_2OH.HCl$ + 0.25M HCl (Shake 30 min.)
		↓
		Soil
		↓
Crystalline Fe oxides Mn	←------------------	50 ml 0.2M $(NH_4)_2$ C_2O_4 + 0.2M $H_2C_2O_4$ (pH 3) + 0.1 M ascorbic acid (30 min. in boiling water bath, Stir occasionally)
		↓
		Soil
		↓
Residual Mn	←------------------	Conc., HF, conc. $HClO_4$ and conc. HCl in sequence

Figure 2: Sequential fractionation scheme for partitioning Mn in soils (Tessier *et al.,* 1979)

(2011) which showed a positive and significant response of wheat to Mn and NO_3^- application. Similarly, Arshad *et al.* (1999) studied the effect of mixed ammonia and nitrate application on nutrition of wheat under different soil salinity regimes under pot culture conditions and found that grain and straw yields were highest with ammonia and nitrate application in the ratio of 50:50. However, balanced N nutrition was helpful to ameliorate the harmful affect of salinity to some extent.

Grain and straw Mn concentration in wheat

Result of the experiment presented in Table 3 revealed that wheat grain and straw Mn concentration increased significantly with increased levels of NO_3^- and Mn application. Mean concentration of Mn in grain increased significantly from 12.76 mg kg^{-1} in control to 14.26, 16.18, 17.33 mg kg^{-1} and in straw the increased ranges from 28.87 mg kg^{-1} in control to 30.25, 30.82 and 32.83 mg kg^{-1} with application of 30, 60 and 90 mg NO_3^- kg^{-1}. The highest concentration of Mn in grain and straw were recorded with 90 mg NO_3^- kg^{-1} addition. Application of 25 and 50 mg Mn kg^{-1} in grain increased significantly Mn content from 8.25 mg kg^{-1} in control to 15.42 and 21.72 mg kg^{-1} and in straw increased significantly from 22.22 mg kg^{-1} in control to 29.80 and 40.06 mg kg^{-1}, respectively. The highest concentration of Mn in grain and straw were also recorded with 50 mg Mn kg^{-1} application. In wheat plant, increasing levels of NO_3^- and Mn application were found to increase Mn content and maximum Mn content were recorded with the application of 90 mg NO_3^- kg^{-1} and 50 mg Mn kg^{-1} soil application. The interaction effect of NO_3^- and Mn on grain and straw Mn content was non significant (Table 3). It is also recognised that application of NO_3^- and Mn during crop growth improves the mineral status of plants and increase the yield of wheat. Similar results were also recorded by Reddy and Reddy (2008) which showed that application of organic manures in conjunction with nitrate fertilizer significantly improved the availability of Mn in soil. Thus, the content of Mn increased with increasing level of nitrate and Mn application. Similarly, Malakauti and Ziaeian (2002) reported that the application of Cu, Zn, Fe and Mn in the presence of NPK caused a significant increased in grain yield, straw yield, 1000 grain weight and the number of seeds per spikelet in wheat. With the application of these nutrients their concentration in grain and flag leaves also increased significantly.

Grain and straw Mn uptake in wheat

Data presented in Table 4 revealed that the Mn uptake by wheat cultivar WH 1105 increased significantly with increased level of NO_3^- and Mn application. The maximum uptake of Mn by wheat crop was recorded at 90 mg NO_3^- kg^{-1} and 50 mg Mn kg^{-1} application. The wheat grain Mn uptake increased significantly from 95.38 μg pot^{-1} in control to 110.75, 132.41, 147.18 μg pot^{-1} and in straw the increase was from 309.02 μg pot^{-1} in control to 349.81, 397.49 and 435.69 μg pot^{-1} with application of 30, 60 and 90 mg NO_3^- kg^{-1}. Application of 25 and 50 mg Mn kg^{-1} in grain increased significantly Mn uptake from 53.18 μg pot^{-1} in control to 140.85, 170.03 μg pot^{-1} and in straw the increased in Mn uptake varied from 253.25 μg pot^{-1} in control to 380.50 and 485.23 μg pot^{-1}, respectively. All the levels of NO_3^- and Mn proved significantly superior over control in respect of Mn uptake by wheat grain and straw. The interaction effect of NO_3^- and Mn on the utilization of manganese was non significant (Table 4) and maximum values were recorded under 90 mg NO_3^- kg^{-1} and 50 mg Mn kg^{-1} treatment. Shankar *et al.* (2014) also reported an increased in Mn uptake with its addition, over recommended doses of fertilizer. Similarly, in a pot experiment Ashok *et al.* (2009) revealed a significant increased in uptake of N, P, K and Mn by wheat with the application of K and Mn. Application of 25, 50 and 100 ppm K, N uptake by wheat grain with 10 and 20 ppm manganese over control were 3.5 and 5.9 per cent and corresponding increases in the N uptake by wheat straw were found 4.4 and 9.5 per cent, respectively. However, the value of Mn uptake obtained at 100 ppm K was significantly higher as compared to control by wheat grains.

Mn pools in soil

Data presented in Table 5 showed that the distribution of Mn among various chemical forms varied significantly in response to changing soil properties. The data on Mn fractionation revealed that all the fractions of Mn i.e. readily soluble Mn {exchangeable (EX)}, carbonates bound (CARB)-Mn, weakly adsorbed Mn {organic matter bound (OM)} and oxide Mn {Mn oxide bound (MnOX), amorphous Fe oxide bound (AFeOX) and crystalline Fe oxide bound (CFeOX)} in soil increased Mn content significantly with the application of NO_3^- and Mn. Maximum was recorded at 90 mg NO_3^- kg^{-1} and 50 mg Mn kg^{-1} soil application. Among all the fractions, the highest concentration of Mn was recorded in RES fraction followed by MnOX > AFeOX > CFeOX > EX > CARB > OM fractions. Organic matter bound (OM) showed a very little amount of Mn content due to the low content of organic carbon in experimental soil. The interaction effect of NO_3^- and Mn was significant on EX-Mn, OM bound-Mn, AFeOX bound-Mn, RES-Mn, Total-Mn while CARB bound-Mn, MnOX bound-Mn, CFeOX bound-Mn were found to be affected non significant (Table 5).

Readily soluble Mn includes that in solution and the easily exchangeable fraction. This fraction is an important source of Mn to plants but, in the same time, its content in soils is known to vary by orders of magnitude within short periods of time and so its level at any particular time may not be well related to plant Mn uptake. Adsorbed forms of soil Mn are held on soil surface by forces ranging from weak electrostatic to strong ligand-exchange bonds and, although not principal forms of soil Mn, they influence plant availability as they are in pseudo-equilibrium with the readily soluble form. Carbonate-bound Mn includes that chemisorbed or coprecipitated with calcite and related carbonate minerals. Plant available Mn is reduced by adsorption or precipitation with carbonates. Oxide-Mn is readily reduced to available forms and is an important source of Mn for plants. Similar results were reported by Randhawa and Singh (1997) and Jaloud *et al.,* (2013) which showed that Mn-exchangeable, weakly adsorbed, moderately adsorbed, strongly adsorbed, associated with organic matter, occluded and bound by carbonates/acid soluble minerals were in very low proportion while most of the total Mn (35.3 to 84.9%) was remain in the residual fraction.

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