



## Antioxidative responses of wheat plants against foliar application of Manganese sulphate grown in degraded soil

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### Abstract

Excess use of chemical fertilization has unbalanced the mineral nutrition in most of the agricultural lands in and is not directly based on plant requirements. The application of foliar application is reported to be the best method to avoid this unbalanced use of different fertilizers among that soil. Manganese is an essential micronutrient for plant growth and also to enhance the yield of different crops. To study this, an experiment with the use of Manganese sulphate as foliar application on Wheat plants to avoid excess use of chemicals fertilizers and their antioxidative responses was performed and the wheat plants were raised in degraded soil collected from degraded lands near alluvial plain of Gomti river, Lucknow. The whole experiment was performed in control environmental conditions in glass house. For the experiment, Wheat (*Triticum aestivum* L. Variety: HD-2967) was taken, and was grown in pots filled with degraded soil. Manganese functions as activator of more than 35 enzymes in different types of crops and it also influences the yield and plant growth by various physiological and biochemical activities. The enzymatic activity of Wheat plants is also depending on the concentration of Manganese sulphate, and it also affects plant growth, yield, and plant biomass. The use of manganese sulphate as a foliar application can influence the different enzymatic activities such as catalase, peroxidase, and ascorbate peroxidase and it also influences the pigment concentration in the Wheat plants. The plant growth and height are also regulated by Manganese sulphate concentration used as a foliar application on plants. The different concentration of Manganese sulphate as a foliar application on Wheat plants was used as-- 00.0  $\mu$ M, 10.0  $\mu$ M, 50.0  $\mu$ M, and 100.0  $\mu$ M. The optimum result was found at 10.0  $\mu$ M of Manganese sulphate foliar application, whereas at 100.0  $\mu$ M of Manganese sulphate foliar supply plant growth was reduced. The enzymatic activity in Wheat plants was found to be optimum at 10.0  $\mu$ M of Manganese sulphate foliar supply and lowest activities was recorded at 00.0  $\mu$ M of Manganese sulphate foliar application.

**Keywords:** Manganese sulphate, degraded soil, chemical fertilizers, foliar application, enzymatic activity

### Introduction

Wheat (*Triticum aestivum* L.) is one the most important and oldest cereal crops on the Earth (Akhtar *et al.*, 2018; Dhaliwal *et al.*, 2019) [6]. Wheat crops mainly contain carbohydrates, proteins, many vitamins, and some essential micro-nutrients (Igrejas and Branlard, 2020) [15]. Across the world, nearly half of the world's population suffers from the deficiency of different types of micronutrients such as Manganese (Mn) and Zinc (Zn) due to consumption of cereal based food (Aziz *et al.*, 2019) [2]. Deficiency of micronutrients is mainly found in developing countries because of reduced mineral nutrition availability in food, including minerals like manganese (Jankowska *et al.*, 2012) [16] and it results falling in health index among developing countries.

Manganese (Mn) is among the most essential micronutrients for plant growth and it plays very important physico-chemical function in different plants. Manganese also participates in different types of enzymatic activities in different plants such as the formation of carbohydrates nitrogen metabolism and phenol biosynthesis (Graham and Webb, 1991 [9]; Marschner, 1995). Manganese plays a very essential role in the formation of pigments and it also participates in oxidation-reduction processes such as electron transport in the photosynthetic pathway. Several other types of antioxidative enzymes activities depend on the application of manganese in plants such as superoxide

dismutase (SOD) and peroxidase, are dependent on the manganese (Reuveni *et al.*, 1997 [30]; Millaleo *et al.*, 2010) [23, 24]. Peroxidase (POD) enzyme acts as the pathogen's resistance in different crop patterns (Graham and Webb 1991 [9]; Heine *et al.*, 2011). Manganese also participates in the formation of controlling the lignin biosynthesis from the activation of certain enzymes involved in shikimic acid and phenylpropanoid pathways (Romheld and Marschner 1991; Marschner 1995).

In Arabidopsis (rock cress), a number 398 enzymes are directly and indirectly predicted to contain Manganese as a metal binding site in plants (The Uni Prot knowledge base). Among the above, more than 20 % of enzymes show experimental evidence to require Manganese as a co-factor. In some other enzymes, Manganese acts as an inter-exchangeable with some other divalent cations such as Calcium (Ca), Cobalt (Co), Copper (Cu), Magnesium (Mg), and Zinc (Zn). In plant's biochemistry, only the oxygen-evolving complex (OEC) of Photosystem II contains the four-manganese atom clusters with one Ca cation and at least one Cl anion, catalyzing the process of oxidation of water to molecular oxygen (Najaf pour *et al.*, 2012).

Manganese is the most essential micronutrient for plants, since it is linked to the plants by the process of hydrolysis in photosystem II, chlorophyll biosynthesis process and it plays an important role in the breakdown of the chloroplast. The absence of Manganese in the soil impacts on the crops

by different types of plant development such as causing interveinal chlorosis on leaf margins, poor root development, and the development of a smaller number of tiller production particularly in Wheat crops (Lu *et al.*, 2004 [22]; Cakmak, 2008 [3]; Alejandro *et al.*, 2020) [1]. In different types of plants, Manganese acts as a catalyst in different enzymatic reactions and it also participates in plant's respiratory processes where it can manage the redox potential process in plant cells under the light and dark phases (Millaleo *et al.*, 2010) [23, 24].

The deficiency of Manganese in different plants severely impacts the plant carbohydrate formation and fertility of pollen grains during the time of grain filling, which results from the crop yield production was reduced (Marschner, 1995). Deficiency of manganese in different crops significantly decreased the photosynthesis reaction, reduced the plant production and it also reduced the dry matter yield (Schmidt *et al.*, 2016 [32]; Rashed *et al.*, 2019). Manganese also plays an important role in some other diverse processes such as chloroplast development (Rohdich *et al.*, 2000; Hsieh *et al.*, 2008) [14], purine and urea catabolism (Werner *et al.*, 2008; Cao *et al.*, 2010), phospholipid biosynthesis process (Collin *et al.*, 1999 [4]; Nowicki *et al.*, 2005) [29], Ca<sup>2+</sup> signaling process (Kim *et al.*, 2003 [18]; Hashimoto *et al.*, 2012), DNA repairing technique (Takahashi *et al.*, 2007 [35]; Szurmak *et al.*, 2008) [34] and the process of histidine biosynthesis (Glynn *et al.*, 2005).

Different plants can easily absorb the Manganese by the foliar application in soluble form (Katyal and Rattan, 2003) [17]. Some recent studies reported that the use of manganese sulphate as of foliar application showed that nutrients supplied would be absorbed and transported from the application point (leaf foliage) to the growing tissues (Nayyar *et al.*, 1985 [28]; Dhaliwal *et al.*, 2021b). The application of Manganese sulphate in degraded soil may enhance the plant growth and grain yield of Wheat crops. Foliar application of manganese sulphate for different plants plays an effective role in enhancing the plant resistance capacity from different diseases; the efficiency of this technique is mainly depending on the application time.

The foliar application of manganese is found more efficient and useful compared to the application of manganese in the soil for plant growth. Some recent studies also suggest the foliar application of manganese in wheat plants significantly increased the yield from 1.44 % to 5.15 % and the concentration of manganese also increased in plant tissues (Dhaliwal *et al.*, 2011) [5]. A recent study shows the positive result of the foliar application of different plants. The foliar application of manganese under field conditions is greatly efficient and the most practical method to enhance the yield of wheat crops as well as the accumulation of manganese in wheat (Nayyar *et al.*, 1990) [27].

## Material and methods

### 1. Manganese treatment and Plant growth

The experiment was conducted in control conditions in a greenhouse. Before sowing wheat seeds were rinsed thoroughly with distilled water and germinated on the moist filter paper in an incubator at 28° C. After the germination, seeds were sowed in pots filled with degraded soil collected from degraded soil area and kept under the control conditions in green house. After the seeds germination was done in each pot, distilled water was supplied to each pot having wheat plant up to the proper seedling growth and

development. After the sowing period of nearly 25 days, when the seedling growth was properly done in every pot, Manganese sulphate supply was started in the form of foliar application in different concentrations such as 00.0 µm, 10.0 µm, 50.0 µm, and 100.0 µm with one control and it was supplied only distilled water. The stock solution which were supplied to each pot with a nutrient solution had the following composition: 4.0 mM KNO<sub>3</sub>, 8.0 mM Ca (NO<sub>3</sub>)<sub>2</sub>, 1.33 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 2.0 mM MgSO<sub>4</sub>, 0.1 mM Fe EDTA, 0.1 µM KCl, 0.33 mM H<sub>3</sub>BO<sub>3</sub>, 1.0 µM ZnSO<sub>4</sub>, 1.0 µM CuSO<sub>4</sub>, 0.33 mM H<sub>3</sub>BO<sub>3</sub>, 0.1 M NaCl and Manganese sulphate at four different level (00.0 µm, 10.0 µm, 50.0 µm, and 100.0 µm) were added in above Hewitt solution for Wheat foliar application. The pH value of the nutrient solution was adjusted in the range of 5.8 to 6.0. The growth and reproductive development of wheat plants after the foliar application of manganese sulphate was observed. The maximum wheat plant growth and minimum plant growth were measured and compared to the control for each of the level of manganese sulphate foliar application. Wheat plants were examined daily to determine the effect of foliar nutrient supply on the growth and reproductive yield of plants and also the effect of deficiency or excess supply of manganese sulphate with their relation to antioxidative enzymes.

### 2. Relative water content and dry matter yield production

The relative water content of the plant's leaves depends on the amount of water absorbed by different parts of the leaves. The relative water content of the leaf was evaluated by Yamasaki and Dillenburg's (1999) [37] method. The fresh weight of the eight leaves of each treatment was measured immediately. The measured leaf samples were immersed in distilled water at room temperature for 24 hours and after 24 hours, turgid weight of the leaves was measured. Afterward, the leaves were dried at 70° C for 72 hours in an oven, and dry weight was measured (Gao, 2000). The relative water content was calculated through the following equation of the leaf sample:

$$RWC = (TW - DW) / (FW - DW) * 100$$

The dry matter yield production of the wheat plants were measured at the time of harvesting. The dry matter yield production of the plants depends on how much dry matter was produced from each part of the plants. The dry matter of root, stem, and leaves are mainly depending on the soil conditions and supply of different nutrients. In this experiment, the dry matter yield of the wheat plants mainly depends on the concentration of manganese sulphate supplied as a foliar application for plant growth.

### 3. Pigments concentration

The analysis of the total concentration of chlorophyll in plant leaves were determined by the pigment extraction methods of plant leaves in 80 % acetone solution stands for 24 hours in the dark condition at 4° C for cooling. Leaves were measured at 5 mg and crushed with the help of mortar and pestle in acetone solution. The chlorophyll and carotenoid pigment concentrations were determined from a spectrophotometer, according to Hiscox and Israelstam (1979) [13].

#### 4. Enzymatic analysis

The leaf sample of each cultivar from the Wheat pot was collected and was homogenized with the help of mortar and pestle by using 6 ml of citrate phosphate buffer (50 mM) of pH 7.0. The homogenized mixture was filtered with the help of muslin cloth and centrifuged at 5500 rpm for 10 minutes. The supernatant was separated from the pellets which were deposited below the centrifuge tube. With the help of filtered supernatant, the activities of different antioxidative enzymes were analyzed.

The activity of Catalase was estimated by an adaptation of

the permanganate titration method prescribed by Euler and Josephson (1927) [7]. The enzymatic reaction was started by using 1 ml of diluted enzyme extract. The catalase enzyme activity was expressed in terms of the breakdown of hydrogen peroxide.

The ascorbate peroxidase enzymatic activity was determined by the method prescribed by Nakano and Asada (1981) [26]. The oxidation reaction in ascorbate peroxidase enzyme was determined by the fall of absorbance per minute at 290 nm after the addition of hydrogen peroxide solution.

**Table 1:** Effect of different concentrations of manganese sulphate supply on growth and dry matter yield of Wheat Plants Grown in degraded soil.

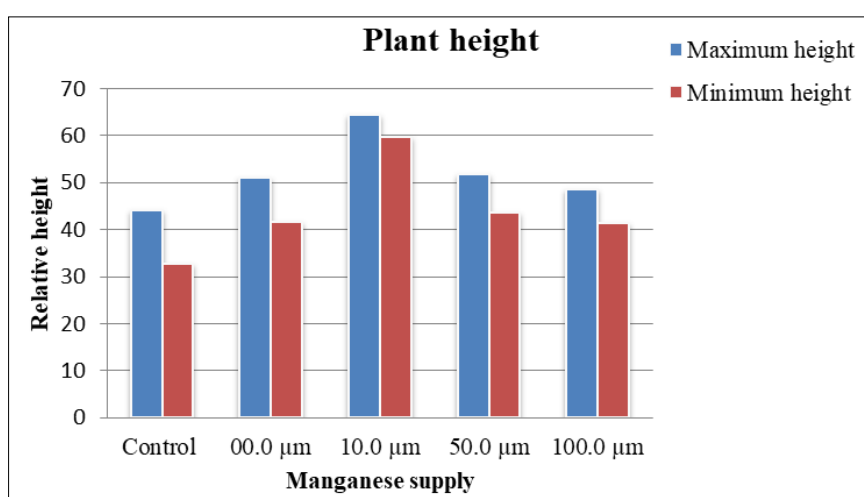
<b>µm of Manganese sulphate supply</b>					
	Control	00.0 µm	10.0 µm	50.0 µm	100.0 µm
<b>Plant height (Cm) After 90 days of foliar application</b>					
Maximum	44.10	51.10+13.69	64.30+31.41	51.70+14.70	48.60+9.259
Minimum	32.80	41.60+21.15	59.80+45.15	43.60+24.77	41.50+20.96
<b>Plant parts</b>					
<b>Dry matter yield (gm plant<sup>-1</sup>)</b>					
Root	0.091	0.139+34.53	0.193+52.84	0.153+40.52	0.132+31.06
Stem	0.360	0.442+18.55	0.544+36.00	0.415+13.25	0.399+9.77
Leaves	0.138	0.177+22.03	0.217+36.40	0.145+4.82	0.125-10.40
<b>Relative water content: %</b>					
Leaves	49.75	54.68+9.01	72.54+31.41	62.50+20.40	46.96-5.94

**Table 2:** Effect of different concentrations of Manganese sulphate supply on chlorophyll concentrations and carotenoids of Wheat plants grown in degraded soil.

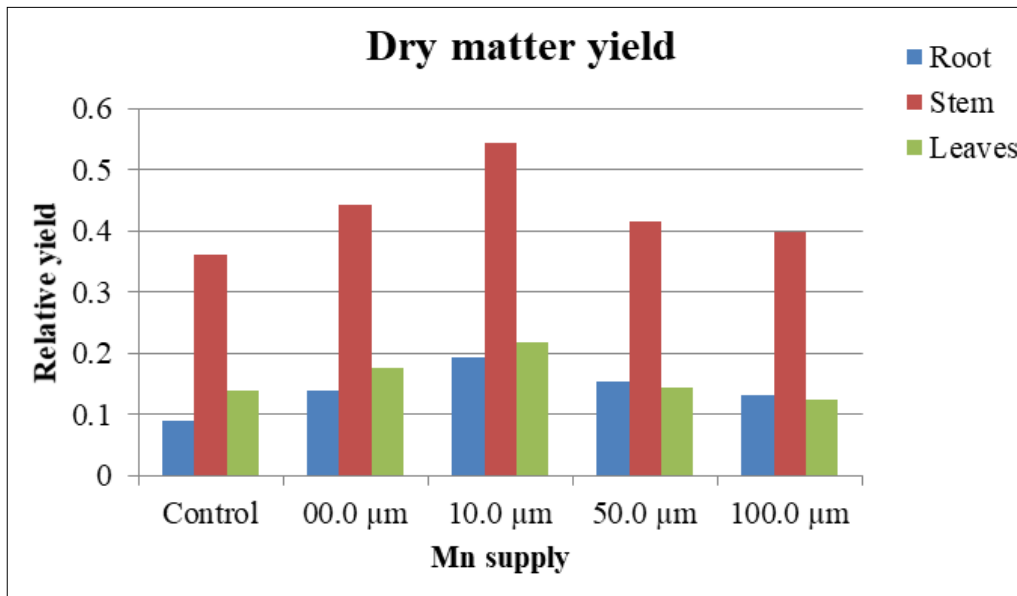
<b>Supply of Manganese sulphate (µm of Mn)</b>					
Plant parts	Control	00.0 µm	10.0 µm	50.0 µm	100.0 µm
<b>Chlorophyll a: mg g<sup>-1</sup> of fresh weight</b>					
Leaves	1.273	1.553+18.02	2.377+46.44	2.122+40.00	1.280+0.54
<b>Chlorophyll b: mg g<sup>-1</sup> of fresh weight</b>					
Leaves	0.866	0.835-3.71	0.925+6.37	0.805-7.57	0.668-29.64
<b>Total Chlorophyll: mg g<sup>-1</sup> of fresh weight</b>					
Leaves	2.135	2.381+10.33	3.252+34.34	2.922+26.93	1.945-9.76
<b>Carotenoid: mg g<sup>-1</sup> of fresh weight</b>					
Leaves	0.649	0.923+29.68	1.089+40.40	0.868+25.23	0.654+0.76

**Table 3:** Effect of different concentration of Manganese sulphate on the activities of Catalase and Ascorbate peroxidase of Wheat plants grown in degraded soil.

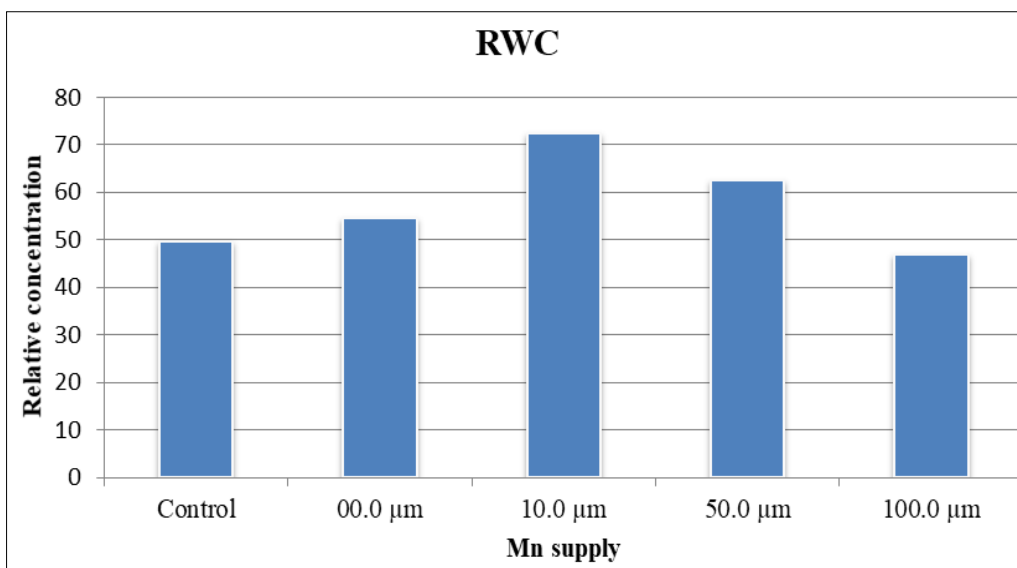
<b>Supply of Manganese sulphate (µm of Mn)</b>					
Plant parts	Control	00.0 µm	10.0 µm	50.0 µm	100.0 µm
<b>Catalase: µmol of H<sub>2</sub>O<sub>2</sub> decomposed mg<sup>-1</sup> of protein</b>					
Leaves	108	120+10.00	128+15.62	116+6.89	96-12.50
<b>Ascorbate peroxidase: µg<sup>-1</sup> of Protein</b>					
Leaves	26.96	32.33+16.60	39.66+32.02	34.55+21.96	27.45+1.78



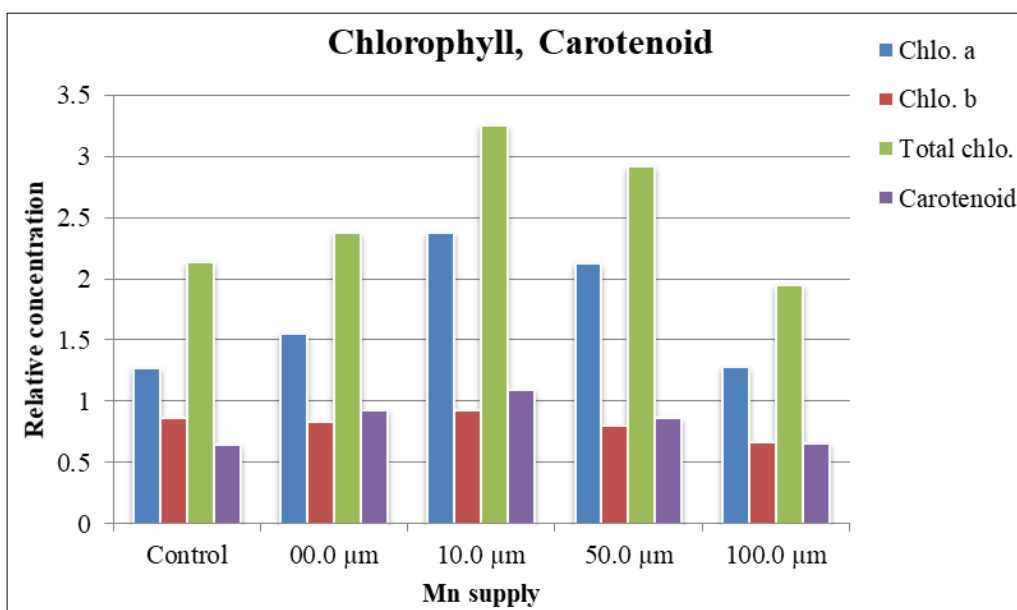
**Graph1:** Representing the relative plant height of wheat plant



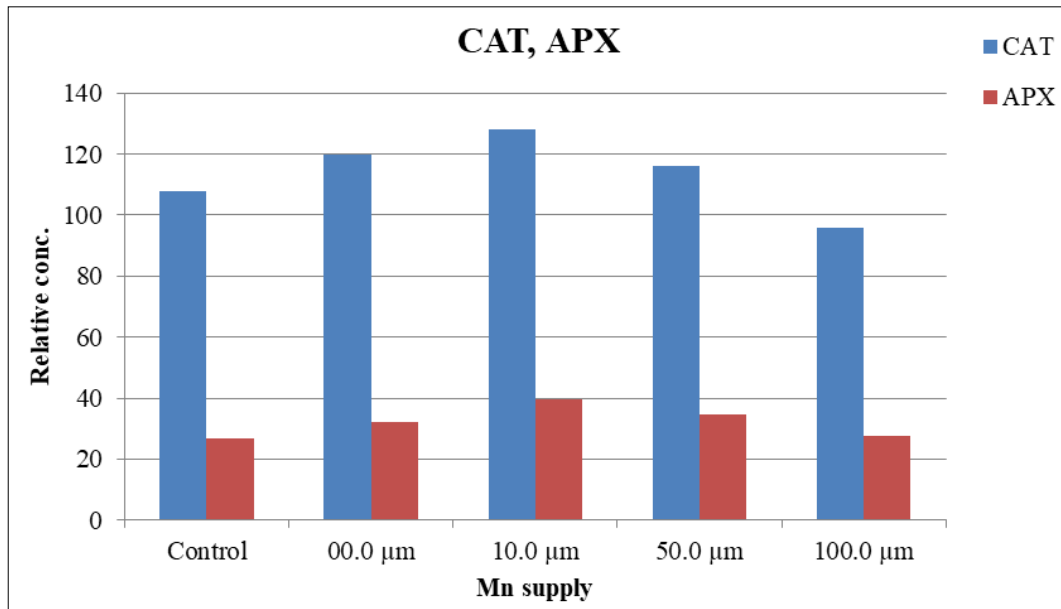
**Graph 2:** Representing the relative dry matter yield of Wheat plants



**Graph 3:** Representing the relative concentration of relative water content of Wheat plants



**Graph 4:** Representing the relative pigments concentration of Wheat plants



**Graph 5:** Representing the relative concentration of catalase and ascorbate peroxidase enzymes of Wheat plants

### Result and discussion

The maximum height of wheat plant (*Triticum aestivum* L. var., HD 2967) was recorded in 10.0 µm of foliar application of manganese sulphate. The wheat plant growth was mainly depended on the soil quality and supply of different concentration of manganese sulphate as foliar application because foliar application may enhance plant's height directly. On the other hand, when 00.0 µm of Manganese sulphate (only the required minerals except manganese) was supplied and the plant growth was compared with control (only distilled water are supplied), it showed 13.69 % of increase and at 10.0 µm supply of manganese sulphate resulted in 31.41 % increase in the plant height. Those plants received manganese sulphate as foliar application in the form of 100.0 µm, resulted only 9.25 % more plant growth compare with control plants.

In this experiment, production of dry matter yield of the wheat plant was reported highest at 10.0 µm of foliar application of manganese sulphate. The quantity of dry matter yield of wheat plants is mainly depends on the application of different nutrients as absorbed by different parts of the plants and soil conditions. The supply of 10.0 µm of foliar application of manganese sulphate in wheat plants, compared with control (only distilled water supplied), the dry matter yield production was significantly higher. The minimum production of dry matter yield was observed at 100.0 µm of manganese sulphate supplied by foliar application.

The relative water content depends on the amount of water absorbed by the plants leaves. The relative water content was optimum at 10.0 µm of foliar application of manganese sulphate. If it compared with control, 31.41 % (highest) content was reported at 10.0 µm of Manganese sulphate as foliar application. The minimum relative water content was recorded at 100.0 µm of manganese sulphate as foliar application to the wheat plants and its value is 5.94 % less compare with control.

The contents of pigment of the wheat plants used manganese sulphate as foliar application for proper growth. In the control solution (only distilled water supplied) pigments concentration was relatively lowest compare with others. The optimum concentration of pigments in wheat

plants was recorded at 10.0 µm of manganese sulphate supply as foliar application and the output of pigment concentration at 10.0 µm of Mn supply as total chlorophyll content was reported 34.34 % highest. At the 100.0 µm of manganese sulphate used as foliar application in wheat plants the total chlorophyll content was relatively 9.76 % less compare with control.

The enzymatic activity of the wheat plants depends on the foliar application of different concentration of Manganese sulphate. Enzymatic activity is mainly depends on the different type of stress imposed on the plants. In this experiment, the catalase enzyme activity depends on the decomposition of hydrogen peroxide during the catalase activity. The activity of catalase enzyme was optimum recorded at 10.0 µm of manganese sulphate supply as foliar application for wheat plant growth, and this activity was comparatively higher with compare to control is 15.62 %. The minimum catalase activity was recorded at 100.0 µm of manganese sulphate supply compared with control is relatively in negative value up to 12.50 %. And at 100.0 µm of manganese sulphate supply as foliar application received lowest catalase activity. On the other hand, ascorbate peroxide (APX) activity in wheat plants is mainly depends on the deposition of protein during the reaction. The ascorbate peroxidase (APX) activity in wheat plants was highest at 10.0 µm of manganese sulphate supplied as foliar application, compare with control 32.02 % more. The lowest APX activity was recorded at 100.0 µm of Mn used as foliar application on wheat plants was reported 1.78 % more compare with control solution.

### Conclusion

The result of this study revealed that the use of manganese sulphate as a foliar application of wheat plants improves the plant height, yield and uptake of manganese in different parts of the plants. The application of manganese sulphate as foliar application in different concentration as 00.0 µm, 10.0 µm, 50.0 µm, and 100.0 µm for wheat plants, 10.0 µm supply of Manganese sulphate is considered as agronomically efficient options for improving growth and yield. Thus, present study revealed the use Manganese sulphate as foliar application may be used to enhance the plant growth

and yield of wheat plants, and the supply of Manganese sulphate as foliar application can improve the antioxidative enzyme mechanism against various stresses and to enhance productivity in various crops.

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