Foliar spraying of MnO₂-NPs and its effect on vegetative growth, production, genomic stability, and chemical quality of the common dry bean

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ABSTRACT
A large part of the income of most developing countries is coming from the agricultural field, where semi of their population relies on farming in their life. The world population is growing rapidly; consequently, there is a requirement to increase agricultural output through the implementation of newfangled technology such as nanotechnology. Herein, the manganese oxide nanoparticles (MnO₂-NPs) were prepared as a foliar nano-fertilizer. The effect of different concentrations (0, 10, 20, 30, and 40 ppm) of MnO₂-NPs on the common dry bean plant criteria, yield, chemical quality of leaves and seeds, genomic DNA, and some genes encoding proteins were investigated, which was planted throughout two sequent seasons 2020 and 2021 in clay soil. The results showed that MnO₂-NPs with a concentration of 30 ppm enhanced the growth criteria and the yield percentage by (45.2 and 48.9%) during two seasons, respectively, of a common dry bean. The chemical quality for leaves and seeds varied in their response to MnO₂-NPs. In addition, the genomic DNA and some genes encoding proteins of the plants were significantly affected by MnO₂-NPs at concentrations of 30 and 40 ppm in comparison to other concentrations.

1. Introduction
The common bean is a fundamental legume in worldwide for straight human consumption. The crop is used fundamentally for its dry seeds, and green pods. In addition, the leaf of the common bean is sometimes utilized as a leaf vegetable and the straw is utilized for animal feed (Shaban et al., 2019; Zewail, 2014). The nutritional value of the common bean is comparatively rich with cheap sources of proteins, carbohydrates, fiber, folic acid, vitamins, and minerals for people in developed and developing countries (Cortés et al., 2013). Moreover, the common bean is described as the only best non-meat source of iron which supplies 23% to 30% of the recommended levels from one serving each day (Shimelis & Rakshit, 2005). Also, it appears an inexpensive source of calcium, and several amino acids as lysine that using in the dietary regimen (El-Mohamudy et al., 2017).

The highest productivity and the best nutritional value of common bean could be achieved via using nutrients, which could be applied in the form of nano-fertilizer. The application of manganese as a fertilizer leads to boosts the productivity and quality of the crop, due to enhancing the plant nourishment and rising photosynthesis in the plants, thus the productivity and goodness increase by improving photosynthetic efficiency (Mousavi et al., 2007). The results of using manganese nanoparticles treatment in mung beans are increased plant growth by about 52% and 38%, for root and shoot, respectively (Pradhan et al., 2013), while the eggplant improved the yield by about 22% (Elmer & White, 2016). This result is due to the small size ranging from 1 to 100 nm, higher particular surface area, and interaction of nano fertilizers that may improve the solubility, expansion, and availability of plants, that being so promoting the yield of plants compared with conventional fertilizers. Where, the essence of nanotechnology is size and control (Abobatta, 2018; Salama et al., 2021a; Shebl et al., 2020).

Manganese is an essential micronutrient, with important functions in the plant as an ingredient of enzymes participatory in photosynthesis and other processes, and plays an important function in photosynthesis, as a structural part of the water-splitting
protein of photosystem II. It acts as an electron store and delivers it to the chlorophyll reaction centers (Millaleo et al., 2010). Moreover, manganese is the section of a major antioxidant frame such as superoxide dismutase that preserves the cells of the plant through inactivating the free radicals that cause spoilage to plants tissues. It is from the above that manganese participates in photosynthesis, respiration, and nitrogen (N) metabolism (Mousavi et al., 2011). Manganese is required necessary in little quantities, which is usually less than 1 kg/ha in cereals and about 2 kg/ha in sugar beets (Draycott & Christenson, 2003). In the same trend, the application of Mn-NPs as a foliar spray to plants is more active practice on plants responses than soil application (Dimkpa et al., 2018). Since the micronutrient is used in a small amount and doesn’t straight contact the soil, which prevents losses during fixation (Abd El-Aziz et al., 2021; Teixeira et al., 2004).

The plants’ biological functions count on the activities that are happening at the molecular scale. Although, the insignificant advance has been made at the molecular scale affected through nanoparticles, which is a fundamental step in estimating probable mechanisms and plants’ effects. Wherefore, it is important to understand plants’ underlying mechanism and its response to nanomaterials, and the drift in gene expression through molecular approaches (Ali et al., 2021).

So, this research aims to investigate the effectiveness of manganese oxide as nanofertilizers on the growth, yield, and quality of common dry bean plants. Additionally, the effect of manganese nanofertilizer on genomic stability of DNA.

2. Material and methods
2.1. Supplies

Seeds of common bean (Phaseolus vulgaris L., cv Nebraska) were purchased from the Agricultural Research Centre, Egypt. The agriculture fertilizer such as potassium sulphate, calcium superphosphate, agriculture sulfur, and ammonium sulfate were bought from Abu Qir Company, Egypt. Manganese nitrate was obtained from Sigma-Aldrich Company.

2.2. Preparation of nanofertilizers

Manganese oxide nanoparticles (MnO2-NPs) were prepared thermally by dissolving 100 g of manganese nitrate (Mn(NO3)2·4H2O) in 50 ml hot water then stirring for 10 min, after that the solution was heated to 100 °C for 24 h in an oven till black viscous liquid was obtained. The deionized water was adding to viscous liquid and centrifugation at 7000 rpm for 15 min. The black precipitate was washed twice with deionized water and then dried at 60 °C (Najafpour et al., 2012).

2.3. Characterization of nanofertilizers

The surface morphology of MnO2-NPs was analyzed using scanning electron microscope (SEM; JEOL/Noran, JSM 6360LV). While transmission electron microscope (TEM; JEM-1230, Japan, magnification 6 × 10^5, and a resolution of 0.2 nm) was used to illustrate the particle size and shape of MnO2-NPs. Where the dispersed solution of the MnO2-NPs was dropped on a carbon-coated copper grid, then was placed in the TEM device after to drying. The X-ray diffraction (XRD) pattern of MnO2-NPs was obtained by using a Diano X-ray diffractometer with radiation source CoKα under operating voltage 45 kV, and a Philips X-ray diffractometer with radiation source CuK (PW 1930 generator, PW 1820 goniometer, and).

2.4. Experiment planning

The experiment was conducted to study the effect of Foliar spraying of MnO2-NPs and its effect on vegetative growth, production, genomic stability, and chemical quality of the common dry bean. The soil had been scrubbed, ploughed, and leveled. In 1st February, it was divided into plots, where each one was 10.5 m². About 47.6 m³/ha of organic manure, 476 kg/ha of calcium superphosphate (15.5% P2O5), 236 kg/ha of agricultural sulfur, 120 kg/ha of potassium sulphate (48% K2O), and 118 kg/ha of ammonium sulphate (20.6% N) were added during the soil preparation. After seeds germination, 236 kg/ha of ammonium sulphate (20.6% N) was added. Before the second irrigation, ammonium sulphate (238 kg/ha; 20.6% N) and potassium sulphate (120 kg/ha, 48% K2O) were applied.

In half of February, about 120 kg/ha of common bean seeds were cultivated throughout two seasons, 2020 and 2021 on the farm with clay texture in El-Menofia governorate, Egypt. The seeds of the
Table 2. Sequences of eight RAPD primers.

| Ser. No. | RAPD primers | Primer sequence (5’-3’) |
|----------|--------------|-----------------------|
| 1        | OP-A18       | AGGTGACCGTG            |
| 2        | OP-B18       | GGACCCTTAC             |
| 3        | OP-B20       | CCACGCGAGT             |
| 4        | OP-P5        | CCCGGTGTAAC            |
| 5        | OP-P9        | GTGGTCCGCA             |
| 6        | OP-P14       | CCAGCGGAC              |
| 7        | OP-P16       | CCAAGCTGCC             |
| 8        | OP-P17       | TGGACCGCCCT            |

common bean were cleaned from dust and odd substances manually, after that it was immersed in water for one hour before planting to encourage the germination of common bean seeds. The seeds were cultivated with rate 2 seeds/hills, and the space between hills on one side of the ridge was 30 cm while between the ridges was 60 cm. The physical and chemical characteristics of the cultivated soil, mean of two seasons, have been analyzed as described by Cottenie et al. (1982) (Table 1).

2.5. Experimentation treatments

After 20 days of sowing common bean plants, they were sprayed with MnO2-NPs at various concentrations (0, 10, 20, 30, and 40 ppm), using hand-pump garden sprayer. Each treatment was sprayed with one liter of suspended nanomaterials in water. The trial layout was a complete randomized block design with three replicates.

2.6. Vegetative growth characteristics

Various vegetative growth criteria such as the length of plant and root, number of leaves, and flowers per plant, as well as fresh and dry weight for plants were studied on five plants that were taken randomly from each treatments in the vegetative stage (45 days of planting).

2.7. Yield and its attributes

The number and weight of pods and seeds per plant, seed index (weight of 100 seeds), weight of seeds (t/ha), shelling percentage, as well as shoot residues (kg/ha) were assessed from five plants that were randomly taken from each plot in harvest stage (90 days after planting).

2.8. Chemical analyzes

2.8.1. Photosynthetic pigments

After 45 days from cultivation, fresh leaves were collected to determine its content of photosynthetic pigments as (mg/g) according to the method mentioned by Salama et al. (2021b). The chlorophyll (a and b) and carotenoid contents were determined by spectrophotometer (SHIMADZU 240 UV/VIS spectrophotometer) at 663 and 644 nm and 452, respectively, after extraction in acetone (85%). The pigment content were expressed as mg/g fresh weight of plant material and was calculated by the equations suggested by Jiang et al. (2018) as follow:

\[
\text{Chl a} = 10.3 \times \text{E}_{663} - 0.918 \times \text{E}_{644} (\mu \text{g/ml})
\]

\[
\text{Chl b} = 19.7 \times \text{E}_{644} - 3.870 \times \text{E}_{663} (\mu \text{g/ml})
\]

\[
\text{Carotenoids} = 4.2 \times \text{E}_{452} - (0.0264 \times \text{Chl a} + 0.426 \times \text{Chl b}) (\mu \text{g/ml}).
\]

2.8.2. Proximate analysis

Fresh leaves and seeds were dehydrated at 70°C in the oven to a constant weight and then grinded in an electric mill for a proximate analysis (Jayant et al., 2018). The content of moisture was obtained by desiccation the leaves and seeds at 105°C till constant weight. The ash content was specified from combustion at 550°C for 6 hours in a muffle furnace. The protein content was measured by the Kjeldahl method (N x 6.25). The fiber was determined by acid digestion (1.25%), followed by alkali digestion (1.25%), then ether extraction. The total energy was qualified using equation described by Nwabueze (2007).

2.8.3. Minerals determination

The milled common bean plant samples (leaves and seeds) were digested with H2SO4-H2O2. The contents of potassium and phosphorous were estimated by spectrophotometer, while zinc, manganese, iron, and copper were determined in digested solutions by atomic absorption (Hao et al., 2019).

2.9. Statistical analysis

The data were statistically analyzed according to Snedecor and Cochran (1989) and the LSD was test at a 5% level.

2.10. DNA extraction and RAPD-PCR analysis

The mutagenicity or genotoxicity was studied by using the RAPD-PCR technique to examine and estimate the genomic variation. This technique is low expensive, rapid, reproducible, and does not require specific knowledge of the DNA sequence (Atienzar & Jha, 2006). The genomic DNA was isolated from a common dry bean by using the GeneJET Plant Genomic DNA Purification Mini Kit Kits (Thermo scientific K0791), and then was quantified by using a NanoDrop 1000 spectrophotometer (Thermo Scientific) according to the method mentioned by Osman et al. (2020). Eight random primers were used to perform RAPD-PCR as mention in Table 2. PCR amplification for isolated DNA was performed in 0.2 ml PCR Eppendorf having 12.5 μl
Dream Taq Green PCR Master Mix 2X (Thermo scientific K1081), 1 μl primer 10 pmol (Metabion, German) and 1 μl Template DNA (50 ng/μL) then completed to 25 μl by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 94°C for 5 min (one cycle) then 94°C for 1 min, 37°C for 45 sec and 72°C for 45 sec (35 cycles) then 72°C for 5 min (one cycle) then held at 4°C. Then 100 bp DNA Ladder H3 RTU (GeneDirex, Cat No. DM003-R500) and 5 μl of DNA amplified PCR product was loaded in each well of agarose and then was placed in 1X TAE buffer (1%) and run at 100 V for about 2 hours. The gel was photographed by gel documentation (Bio-Rad) and was analyzed by the Total Lab program to find out the molecular size of each band.

2.11. Protein banding patterns

SDS-PAGE is widely used to describe the genetic structure of crop and to provide information about genes structure (Chandra et al., 2013). It was performed according to the method of Laemmlli (1970). The water-soluble proteins (W.S.P.) were extracted from the leaves samples of randomly selected shoots at seedling and flowering stages. The marker of used protein was BLUltra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500). The results were analyzed by the Total Lab program to obtain the molecular weight of each band.

2.12. Toxicity test

The seeds of common beans were dried in an oven at 105°C till constant weight after that they were grinded to a fine powder. An extraction was obtained from the powder of common bean in water at two concentrations 0.1% and 1.0%. Then Microtox analyzer 500 (USA) was used to measure the toxicity of the samples (Johnson, 2005).

3. Results and discussion

3.1. Characteristics of MnO₂-NPs

The morphological structure of the prepared nanoparticles was illustrated in Figure 1(a) and 1(b). The TEM diagram illustrated a regular diamond shape of MnO₂-NPs as presented in Figure 1(a). In addition, it can be demonstrated that the prepared nanomaterials have particles size less than 100 nm. The SEM image (Figure 1a and 1b) supported this observation, since the particles are gathering to form forming a clear parallelepiped rectangles structure. The crystal structure of the MnO₂-NPs was examined using X-ray diffraction measurements (Figure 1c). The main peaks at 2θ equal 28.6°, 37.3°, 40.9°, 42.8°, 56.6°, 59.3°, and 72.3° are corresponding to plane 110, 011, 020, 111, 121, 220, and 031, respectively. These diffraction peaks resembled the reported values of Reference code 98-005-6006 of MnO₂, where the crystal system is Tetragonal, a space group is P 42/m.
n m, and the mineral name is Pyrolusite (Devaraj & Munichandraiah, 2008; Dewi & Yulizar, 2020).

### 3.2. Vegetative growth characters

Table 3 illustrated that vegetative growth characteristics of common bean plant as expressed as the length of plant and root, number of leaves, and branches per plant, plus the fresh and dry weight of the plant, showed a significant increase with increasing MnO$_2$-NPs concentrations compared with the control during two successive seasons 2020 and 2021. The concentration 40 ppm of MnO$_2$-NPs enhanced the percentage of plant length by (13.9 and 14.8%) and root length by (81.3 and 76.5%) during two seasons, respectively. Conversely, the increasing in the number percentage of leaves was (42.8 and 42.4%), while flowers number was (39.4 and 39.5%) per plant, furthermore the increasing in the percentage of fresh weight was (69.7 and 69.8%) and dry weight was (59.3 and 52.6%) of common bean plants recorded with the 30 ppm of MnO$_2$-NPs during two seasons, respectively. As reported before by Pradhan et al. (2013) recorded that the mung beans plant treated with manganese nanoparticles led to increasing the growth of roots by about 52%, shoots by about 38%, and the number of rootlets by about 71% at a concentration of 0.05 mg/l. In another study, Liu et al. (2016) found that MnO$_x$-NPs about 71% at a concentration of 0.05 mg/l. In shoots by about 38%, and the number of rootlets by increasing the growth of roots by about 52%, beans plant treated with manganese nanoparticles even at the higher concentration of 50 mg/l. Over lettuce seeds by about 63% as compared to control slightly minimized the germination percentage of by Pradhan et al. (2013) recorded that the mung bean plants recorded with the 30 ppm of MnO$_2$-NPs and dry weight was (59.3 and 52.6%) of common bean plant. Additionally, manganese has a significant function in stimulating the enzymes for the metabolism of carbohydrates and phosphorylation. Consequently, it leads to the enhancement of the efficient quantitative properties of the common bean yield by the systematization of the plant’s growth. Therefore, the implementation of Mn is major for gaining the maximum profitable yield Nadergoli et al. (2011). Likewise, Ruttkay-Nedecky et al. (2017) illustrated that manganese is an fundamental micronutrient for growth regulation and plants development. Elmer and White (2016) found that treating eggplant plants with manganese nanoparticles improved productivity by 22% compared to control. Also, the application of Mn-NPs as foliar with a concentration 20 ppm gave the highest yield and the best characteristics of the fruits of the squash plant compared with other treatments (Shebl et al., 2019).

These results can be expounded on the basis of the effect of MnO$_2$-NPs on the genomic DNA of plants, where the most effective concentrations of

| Plant length (cm) | Root length (cm) | No. leaves/plant | No. flowers/plant | Fresh plant weight (g) | Dry plant weight (g) |
|-------------------|-----------------|------------------|------------------|------------------------|---------------------|
| Control           | 53.3 ± 2.8      | 53.7 ± 3         | 12.3 ± 2.0       | 13.2 ± 1.5             | 28.0 ± 2.6          |
| 10 ppm            | 52.0 ± 2.2      | 53.0 ± 2         | 14.7 ± 2.1       | 15.3 ± 2.5             | 28.0 ± 3.0          |
| 20 ppm            | 55.7 ± 1.7      | 56.3 ± 3.3       | 16.0 ± 1.0       | 17.3 ± 1.5             | 34.0 ± 2.0          |
| 30 ppm            | 56.7 ± 1.3      | 57.3 ± 1.6       | 21.3 ± 2.0       | 21.7 ± 0.9             | 40.0 ± 3.5          |
| 40 ppm            | 60.7 ± 1.5      | 61.7 ± 1.5       | 22.3 ± 2.3       | 23.3 ± 2.0             | 66.0 ± 2.0          |
| Control           | 53.3 ± 2.8      | 53.7 ± 3         | 12.3 ± 2.0       | 13.2 ± 1.5             | 28.0 ± 2.6          |
| 10 ppm            | 52.0 ± 2.2      | 53.0 ± 2         | 14.7 ± 2.1       | 15.3 ± 2.5             | 28.0 ± 3.0          |
| 20 ppm            | 55.7 ± 1.7      | 56.3 ± 3.3       | 16.0 ± 1.0       | 17.3 ± 1.5             | 34.0 ± 2.0          |
| 30 ppm            | 56.7 ± 1.3      | 57.3 ± 1.6       | 21.3 ± 2.0       | 21.7 ± 0.9             | 40.0 ± 3.5          |
| 40 ppm            | 60.7 ± 1.5      | 61.7 ± 1.5       | 22.3 ± 2.3       | 23.3 ± 2.0             | 66.0 ± 2.0          |
| Control           | 53.3 ± 2.8      | 53.7 ± 3         | 12.3 ± 2.0       | 13.2 ± 1.5             | 28.0 ± 2.6          |
| 10 ppm            | 52.0 ± 2.2      | 53.0 ± 2         | 14.7 ± 2.1       | 15.3 ± 2.5             | 28.0 ± 3.0          |
| 20 ppm            | 55.7 ± 1.7      | 56.3 ± 3.3       | 16.0 ± 1.0       | 17.3 ± 1.5             | 34.0 ± 2.0          |
| 30 ppm            | 56.7 ± 1.3      | 57.3 ± 1.6       | 21.3 ± 2.0       | 21.7 ± 0.9             | 40.0 ± 3.5          |
| 40 ppm            | 60.7 ± 1.5      | 61.7 ± 1.5       | 22.3 ± 2.3       | 23.3 ± 2.0             | 66.0 ± 2.0          |

Three replicates were used.

### 3.3. Common bean yield

Yield and its components of common bean plants are shown in Table 4 during the two seasons of agriculture 2020 and 2021. The data showed that MnO$_2$-NPs appeared a significant effect on the common bean yield and its components. The common bean plants treated with 30 ppm MnO$_2$-NPs had a higher number and weight of seeds/plant, also weight of pods/plant, as well as the yield (t/ha). Where the yield percentage increased by (45.2 and 48.9%) during two seasons, respectively. Contrariwise, the highest value of the number of pods/plant, seed index, and weight of residues per plant and per hectare appeared with plants sprayed by 40 ppm MnO$_2$-NPs, while the best shelling percentage was recorded by using the lowest concentration of MnO$_2$-NPs (10 ppm). Where the shelling percentage increased by (18.0 and 15.3%) during two seasons, respectively. The increase in yield was due to the improvement of vegetative growth characteristics when spraying plants with MnO$_2$-NPs concentration (30 ppm), as shown in Table 3. This effect shows that the MnO$_2$-NPs concentration (30 ppm) is enough to enhancement the translocation of the metabolites from vegetative growth to the seeds, which reflected in the improvement of the seed yield of the common bean plant. Additionally, manganese has a significant function in stimulating the enzymes for the metabolism of carbohydrates and phosphorylation. Consequently, it leads to the enhancement of the efficient quantitative properties of the common bean yield by the systematization of the plant’s growth. Therefore, the implementation of Mn is major for gaining the maximum profitable yield Nadergoli et al. (2011). Likewise, Ruttkay-Nedecky et al. (2017) illustrated that manganese is an fundamental micronutrient for growth regulation and plants development. Elmer and White (2016) found that treating eggplant plants with manganese nanoparticles improved productivity by 22% compared to control. Also, the application of Mn-NPs as foliar with a concentration 20 ppm gave the highest yield and the best characteristics of the fruits of the squash plant compared with other treatments (Shebl et al., 2019).
MnO₂-NPs on the plant genome were 30 and 40 ppm in contrast with other concentrations. Because some bands have vanished and others were appeared with these concentrations compared with the control, as shown in Table 8. Also, the improvement in the vegetative growth criteria such as the number of leaves, branches, and flowers per plant, Table 3, may be the reason for the enhancement of the yield and its components of common bean plants.

Furthermore, many studies illustrate the effect of Mn in microscale on productivity. Teixeira et al. (2004) found that the application of Mn on the leaves of the common bean by 315 g/ha increases the yield by 34%, while its combination with Zn (280 g/ha) increases the yield by 60%. Herein, it can be found that 30 ppm of MnO₂-NPs (≈14 g/ha) enhanced the yield percentage by about 47%. This indicates the possibility of reducing the quantity of traditional fertilizer used and at the same time reaching a higher productivity.

### 3.4. Chemical studies

#### 3.4.1. Photosynthetic pigments

The capability of plants to photosynthesis, as well as the rate of photosynthesis, is due to their content of photosynthetic pigments (chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and carotenoids). Table 5 illustrated that the photosynthetic pigments content in common bean leaves were affected with different concentrations of MnO₂-NPs, during the two growing seasons 2020 and 2021. The content of Chl a, Chl b, total chlorophyll, and carotenoids were increased in common bean plants treated with 30 ppm MnO₂-NPs as compared to other concentrations. This may be due to that manganese is involved in photosynthesis, respiration, and nitrogen metabolism (Dimkpa et al., 2018). Furthermore, Ruttkay-Nedecky et al. (2017) showed that manganese plays a central role in oxygen photosynthesis, either directly or indirectly. A similar result was found before by Jhanji et al. (2014) they found that the chlorophyll content in wheat plants increased when plants were treated with manganese. Shebl et al. (2019) recorded that the foliar application of manganese oxide NPs with a concentration of 20 ppm was enough to enhance the photosynthetic pigments content in the leaves of squash plant as compared to other elements. On the contrary, the chlorophyll content was reduced by using a high concentration of manganese by about 378 mg/l. Pradhan et al. (2013) found that the chloroplasts treated with Mn-NPs showed greater photophosphorylation, and oxygen evolution compared with MnSO₄-treated chloroplasts, which were determined either by biophysical or biochemical techniques.
Additionally, Eaton indicated that manganese is remarkable for the composition of carotenoids, sterols, and gibberellic acid seeing that it catalyzes phytoene synthetase.

### 3.4.2. Proximate components

It is evident from (Table 6) that the leaves of the common bean plant treated with various concentrations of manganese nanoparticles resulted in significant increment on proximate components, during two sequential seasons 2020 and 2021. The content of moisture in leaves showed the highest with plants sprayed with a concentration of 40 ppm MnO$_2$-NPs. A concentration of 30 ppm MnO$_2$-NPs recorded the best percentage of protein and ash. In contrast, energy content significantly increased by application MnO$_2$-NPs with a concentration of 20 ppm. In contrast, the percentage of carbohydrates, lipids, and ash was increased with traditionally grown common bean plants. This result might be due to that the proximate components were varied in their response to MnO$_2$-NPs concentration. Where Schmidt et al. (2016) showed that manganese has an important role in the photosystem, respiration, and nitrogen assimilation as a catalyst. Also, Shebl et al. (2019) found that the application of 20 ppm Mn-NPs as foliar fertilizer recorded the best organic matter and protein percentage, as well as the highest total energy in the leaves of the squash plant. From the previous results, the application of nanomaterials boosted the nutritional values of plants as a result of ameliorating crop yields (Adisa et al., 2019; Salama et al., 2019).

The data in Table 6 revealed that the proximate components of seeds common bean were affected with several concentrations of manganese nanoparticles, during two consecutive seasons 2020 and 2021. The marked increase of moisture percentage in seeds was observed with a 30 ppm MnO$_2$-NPs. While the highest carbohydrates percentage showed in plants spraying with a 20 ppm MnO$_2$-NPs. On the contrary, a concentration of 10 ppm MnO$_2$-NPs gave the maximum energy percentage. Also, Fat, fiber, and ash are increased when common bean plants are grown conventionally. This result might be due to the proximate components varied in their response to MnO$_2$-NPs concentration. Verma et al. (2021) found that the impact of plant species relies on the concentrations of nanoparticles. Furthermore, nano-fertilizers have a substantial part in the physiological and biochemical processes of crops through raising the availability of nutrients, that support to reinforce metabolic processes and photosynthesis (Adisa et al., 2019).

### 3.4.3. Minerals content

The results in Table 7 illustrated that the minerals content of leaves and seeds were significantly affected by spraying MnO$_2$-NPs as a foliar application on the common bean plant, during two sequential seasons 2020 and 2021. The best value of nitrogen, phosphorus, potassium, and zinc was recorded with leaves of common bean treated with MnO$_2$-NPs concentration (30 ppm). Reciprocally, 40 ppm MnO$_2$-NPs appeared the highest content of manganese, iron, and copper as compared to concentrations and control. This effect shows that the content of minerals was significantly increased with raising the MnO$_2$-NPs concentration. Alike results were recorded by Shebl et al. (2019), who indicated that the nitrogen percentage was increased in squash leaves when plants were sprayed with Mn-NPs at a concentration of 20 ppm.

As for seeds of common bean in Table 7, the percentage of nitrogen showed the best content with a 10 ppm concentration of MnO$_2$-NPs. On the other hand, the phosphorus and potassium percentage gave the highest with a concentration of 30 ppm MnO$_2$-NPs. Instead, the seeds obtained from plants treated with 40 ppm MnO$_2$-NPs showed the better content of zinc, manganese, iron and copper compared to other concentrations in both seasons of cultivation. Regarding the copper content of bean seeds, the concentration of 20 ppm showed the best copper content.

The effect of MnO$_2$-NPs concentrations on the content of minerals in the common bean leaves and seeds can be attributed to the function of the manganese in the metabolic processes and breakthrough the plant cell (Dimkpa et al., 2018; Schmidt et al., 2016). Also, manganese being applied to plants has

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**Table 5.** Effect of MnO$_2$-NPs concentrations on physiological pigments of common leave through successive seasons 2020 and 2021.

| Treatment | Chl a (mg/g fresh weight) | Chl b (mg/g fresh weight) | Total chlorophyll (mg/g fresh weight) | Carotenoids (mg/g fresh weight) |
|-----------|---------------------------|---------------------------|-------------------------------------|-------------------------------|
|           | 2020  | 2021  | 2020  | 2021  | 2020  | 2021  | 2020  | 2021  | 2020  | 2021  | 2020  | 2021  |
| Control   | 0.06 ± 0.008 | 0.06 ± 0.001 | 0.11 ± 0.010 | 0.13 ± 0.005 | 0.17 ± 0.009 | 0.19 ± 0.006 | 0.15 ± 0.005 | 0.16 ± 0.010 |
| 10 ppm    | 0.04 ± 0.003 | 0.05 ± 0.009 | 0.10 ± 0.004 | 0.12 ± 0.011 | 0.14 ± 0.007 | 0.17 ± 0.014 | 0.14 ± 0.004 | 0.15 ± 0.009 |
| 20 ppm    | 0.06 ± 0.005 | 0.08 ± 0.01 | 0.14 ± 0.004 | 0.16 ± 0.010 | 0.20 ± 0.009 | 0.24 ± 0.001 | 0.16 ± 0.003 | 0.17 ± 0.015 |
| 30 ppm    | 0.07 ± 0.004 | 0.09 ± 0.005 | 0.16 ± 0.004 | 0.17 ± 0.010 | 0.23 ± 0.008 | 0.26 ± 0.005 | 0.18 ± 0.002 | 0.19 ± 0.006 |
| 40 ppm    | 0.05 ± 0.004 | 0.06 ± 0.002 | 0.13 ± 0.01 | 0.15 ± 0.005 | 0.18 ± 0.012 | 0.21 ± 0.003 | 0.17 ± 0.003 | 0.15 ± 0.005 |
| LSD 5%    | 0.006 | 0.008 | 0.007 | 0.011 | 0.008 | 0.009 | 0.004 | 0.013 |

Three replicates were used.
a great effect on the multifariousness biological systems inclusively photosynthesis, respiration, and nitrogen absorption (Mousavi et al., 2011). It can be concluded that the increment in nutrients can be referred to as the increased production associated with an enhancement in nutrients at a higher nutritional supplements concentration in seed and other parts of the plant (Nagamani et al., 2020). Because, the nanoparticles interactions are by the absorption, transfer, and accumulation in the plant species (Rico et al., 2011).

### 3.5. Effect of MnO₂-NPs on genomic DNA using RAPD-PCR marker

The deviation in the DNA fingerprints of common dry bean plants, which were sprayed with different concentrations of MnO₂-NPs (0, 10, 20, 30, and 40 ppm), were estimated by using eight RAPD primers (Figure 2 and Table 8).

The primers OP-B18, OP-B20, and OP-P17 showed seven, ten, and eight bands ranged from 2780 to 330, 1770 to 100, and 1790 to 385 bp, respectively. These primers hadn’t any polymorphic band. While the primer OP-P9 displayed nine bands with eight monomorphic bands and one polymorphic band at 1750 bp (Rf 0.409), which were present only in the treatment 20, 30, and 40.

Primer OP-A18 showed seventeen bands ranged from 1400 to 155 bp, with twelve monomorphic and five polymorphic. Where one (+) unique band and one (-) unique were obtained at molecular size 1955 bp (Rf 0.449) and 1550 bp (Rf 0.497), respectively, in 40 ppm MnO₂-NP treatment. Otherwise the bands at 1260 bp (Rf 0.532) and 500 bp (Rf 0.732) were disappeared only in control. The band at 1665 bp (Rf 0.477) appeared only in 30 and 40 ppm treatments.

Primer OP-P5 showed one monomorphic band and six polymorphic bands ranged from 1485 to 530 bp. Two (+) unique bands (660 bp and 530 bp) and two – (-) unique bands (1485 bp and 1300 bp) were shown in control only. In addition, the band at 1125 bp (Rf 0.526) was disappeared in 40 ppm treatment while the band at 1020 bp (Rf 0.538) disappeared in 30 and 40 ppm treatment.

Primer OP-P14 showed nine bands with three monomorphic bands and six polymorphic bands ranged from 1900 to 440 bp. Only the control had one (+) unique bands at 440 bp (Rf 0.720). While

### Table 6. Effect of MnO₂-NPs on proximate components of the common bean during two successive seasons 2020 and 2021.

| Treatment | Moisture % | Carbohydrate % | Protein % | Lipid % | Fiber % | Ash % | Total energy (kcal/g) |
|-----------|------------|----------------|-----------|---------|---------|-------|----------------------|
|           | 2020       | 2021           | 2020      | 2021    | 2020    | 2021  | 2020                | 2021    |
| Leaves    |            |                |           |         |         |       |                     |
| Control   | 7.33       | 7.37           | 39.5      | 39.6    | 18.2    | 18.3  | 4.73                | 4.80    |
| 10 ppm    | 6.26       | 6.43           | 37.9      | 37.4    | 20.0    | 19.7  | 4.31                | 4.40    |
| 20 ppm    | 6.46       | 6.57           | 38.4      | 38.1    | 22.7    | 22.6  | 4.33                | 4.40    |
| 30 ppm    | 6.76       | 7.05           | 33.7      | 33.6    | 23.9    | 23.7  | 4.31                | 4.36    |
| 40 ppm    | 7.50       | 8.00           | 35.4      | 35.9    | 21.2    | 21.4  | 4.55                | 4.62    |
| LSD 5%    | 0.27       | 0.36           | 0.26      | 0.37    | 0.36    | 0.26  | 0.13                | 0.08    |
| Seeds     |            |                |           |         |         |       |                     |
| Control   | 6.4        | 6.3            | 39.2      | 39.3    | 20.2    | 20.2  | 4.00                | 3.90    |
| 10 ppm    | 7.0        | 7.2            | 56.9      | 57.2    | 24.0    | 24.0  | 1.54                | 1.51    |
| 20 ppm    | 8.8        | 8.9            | 59.9      | 59.5    | 19.6    | 19.5  | 1.25                | 1.24    |
| 30 ppm    | 10.3       | 10.2           | 57.9      | 57.8    | 21.3    | 21.2  | 0.92                | 0.93    |
| 40 ppm    | 8.2        | 8.3            | 57.2      | 57.3    | 22.9    | 22.6  | 0.54                | 0.57    |
| LSD 5%    | 0.12       | 0.26           | 0.80      | 0.80    | 2.32    | 1.25  | 0.15                | 0.08    |

### Table 7. Effect of MnO₂-NPs on minerals of common bean leaves and seeds during two successive seasons 2020 and 2021.

| Treatment | N % | P % | K % | Zn ppm | Mn ppm | Fe ppm | Cu ppm |
|-----------|-----|-----|-----|--------|--------|--------|--------|
|           | 2020 | 2021 | 2020 | 2021   | 2020   | 2021   | 2020   |
| Leaves    |      |      |      |        |        |        |        |
| Control   | 2.96 | 2.98 | 0.25 | 0.22   | 1.35   | 1.32   | 15.5   |
| 10 ppm    | 3.25 | 3.20 | 0.29 | 0.27   | 1.55   | 1.48   | 16.4   |
| 20 ppm    | 3.69 | 3.67 | 0.39 | 0.34   | 1.66   | 1.59   | 18.1   |
| 30 ppm    | 3.89 | 3.85 | 0.40 | 0.37   | 2.25   | 2.12   | 22.7   |
| 40 ppm    | 4.15 | 4.14 | 0.43 | 0.39   | 2.71   | 2.60   | 27.2   |
| LSD 5%    | 0.74 | 0.83 | 0.05 | 0.02   | 0.15   | 0.13   | 0.31   |
| Seeds     |      |      |      |        |        |        |        |
| Control   | 3.28 | 3.28 | 0.33 | 0.31   | 1.20   | 1.13   | 20.1   |
| 10 ppm    | 3.90 | 3.85 | 0.35 | 0.32   | 1.23   | 1.22   | 22.6   |
| 20 ppm    | 3.46 | 3.45 | 0.46 | 0.44   | 2.51   | 2.37   | 24.6   |
| 30 ppm    | 3.72 | 3.67 | 0.45 | 0.42   | 2.08   | 2.03   | 25.6   |
| 40 ppm    | 4.15 | 4.14 | 0.43 | 0.39   | 2.71   | 2.60   | 27.2   |
| LSD 5%    | 0.16 | 0.47 | 0.05 | 0.03   | 0.11   | 0.10   | 1.28   |

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Table 8. The Influence of MnO$_2$-NPs on genomic DNA of the common bean by RAPD-PCR.

| Ser. No. | RAPD primers | Total number of bands | Allele size range (bp) | Mono-morphic bands | Poly-morphic bands | (+-ve) Unique bands | (-ve) Unique bands | Polymorphism percentage | Molecular Size (bp) | Rf cont 10 ppm 20 ppm 30 ppm 40 ppm |
|----------|---------------|-----------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|------------------------|-------------------|----------------------------------|
| 1        | OP-A18        | 17                    | 1955–135              | 12                | 5                 | 1                 | 3                 | 29.41%                 | 1955              | 0.449 0.477 0.497 0.532 0.732 |
|          |               |                       |                       |                   |                   |                   |                   |                        |                   | /C0 /C0 /C0 /C0 /C0 |
| 2        | OP-B18        | 7                     | 2780–330              | 7                 | 0                 | 0                 | 0                 | 0%                     | –                 | – – – – – – – |
| 3        | OP-B20        | 10                    | 1770–100              | 10                | 0                 | 0                 | 0                 | 0%                     | –                 | – – – – – – – |
| 4        | OP-P5         | 7                     | 1485–530              | 1                 | 6                 | 2                 | 3                 | 85.71%                 | 1485              | 0.466 0.480 0.526 0.538 0.646 |
|          |               |                       |                       |                   |                   |                   |                   |                        |                   | /C0 /C0 /C0 /C0 /C0 |
| 5        | OP-P9         | 9                     | 1750–280              | 8                 | 1                 | 0                 | 0                 | 11.11%                | 1750              | 0.409 0.454 0.480 0.508 0.542 |
| 6        | OP-P14        | 9                     | 1900–350              | 3                 | 6                 | 1                 | 4                 | 66.66%                | 1900              | 0.406 0.454 0.480 0.508 0.542 |
|          |               |                       |                       |                   |                   |                   |                   |                        |                   | /C0 /C0 /C0 /C0 /C0 |
| 7        | OP-P16        | 14                    | 1845–100              | 10                | 4                 | 1                 | 2                 | 28.57%                | 1500              | 0.493 0.519 0.538 0.720 0.946 |
|          |               |                       |                       |                   |                   |                   |                   |                        |                   | /C0 /C0 /C0 /C0 /C0 |
| 8        | OP-P17        | 8                     | 1790–385              | 8                 | 0                 | 0                 | 0                 | 0%                     | –                 | – – – – – – – |
| Total    |               | 78                    | 81                    | –                 | 59                | 22                | 5                 | 12                     | 7375              | 10.125 7.375 2.75  |
| Average  |               | 9.75                  | 10.125                | –                 | 7.375             | 2.75              | –                 | –                      | –                 | – – – – – – – |

+-: present, --: absent.
the band at 1000 bp (Rf 0.542) appeared in 20 and 40 ppm.

Finally, the primer OP-P16, had fourteen bands ranged from 1845 to 100 bp with ten monomorphic bands and four polymorphic bands. Since two – ve unique bands at 1320 bp (Rf 0.519) and 120 bp (Rf 0.934) disappeared only in 40 ppm treatment, but it had one + ve unique at 100 bp (Rf 0.945). while the band at 1500 bp (Rf 0.493) was absent in treatments 30 and 40 ppm.

It data illustrated that the concentrations 30 and 40 ppm of MnO2-NPs were more effective on the plant genome compared to the control, where some bands were disappeared and others were appeared in those treatments. This illustrated that this concentration could acting as the genotoxic agent or DNA mutation in the priming sites. Atienzar and Jha (2006) stated that the variation in the priming sites performs a change in the annealing conditions and homologous recombination that causes the disappearance of certain bands and the appearance of new bands. Also, Ghosh et al. (2016) found that ZnO-NPs at a high concentration causes losing of membrane integrity for root meristem of Allium cepa cells, and increasing of chromosome aberrations, as well as breaking of DNA strand.

The mutagenic effect of MnO2-NP could be qualitatively measured by genomic template stability

Figure 2. The effect of MnO2-NPs concentrations on the genomic DNA for common dry bean by RAPD-PCR. M: DNA ladder 100 bp, 1: control, 2: 10 ppm, 3: 20 ppm, 4: 30 ppm, 5: 40 ppm.
MnO2-NPs concentrations had an influence on the foliar spraying concentration of Ag-NPs up to 80 ppm on tomato plant (Çekic et al., 2017), ZnO-NPs up to 50 ppm on bean plant (Mahmoud, 2019), and MoO3-NPs up to 30 ppm on Phaseolus vulgaris plants (Osman et al., 2020), which was in agreement with our results.

### 3.6. Protein profile

Figure 3 illustrates the effect of MnO2-NPs on the genome of the common dry bean, where the expression of some genes encoding proteins was switch on or off. Twenty-three bands wear appeared in the seedling stage, where fifteen bands were monomorphic and eight bands were polymorphic. The number of proteins bands in the control was 22 but in treatment 10 and 20 ppm MnO2-NP was 21 bands while in treatment 30 and 40 ppm were 17 and 16 bands, respectively. The protein with Mw 110 kDa displayed in all treatments except 40 ppm MnO2-NP, but the proteins with Mw 85, 72, 63, and 32 kDa were absent in the case of treatments 30 and 40 ppm. Whereas the protein with Mw 28 kDa was truant in the control but formed under all MnO2-NP treatments.

On the other hand, twenty-six bands obtained in the flowering stage, where twenty-one bands were monomorphic and five bands were polymorphic (Table 10). The protein with Mw 110 kDa was present in all treatments except treatment 40 ppm, while proteins with Mw 100 and 72 kDa were truant from the plants that treated with 30 and 40 ppm MnO2-NP. In addition, the proteins with Mw 100, 94, and 68 kDa obtained in the flowering stage but disappeared in all MnO2-NP treatments. Some metabolic processes in plants had been affected by nanoparticles application (Hossain et al., 2015; Nair & Chung, 2015).

| Table 9. Effect of MnO2-NPs on a number of new appeared (+) and disappeared (−) bands as related to control and Genome Template Stability percentage (GTS%) in common bean plants under the effect of using eight RAPD primers. |
|---|---|---|---|---|---|---|
| **Treatments** | **10 ppm** | **20 ppm** | **30 ppm** | **40 ppm** | **Control** |
| **Primer name** | **1550 bp** | **1550 bp** | **1550 bp** | **1550 bp** | **1550 bp** |
| **Ser. No.** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Primer name** | **OP-A18** | **OP-B18** | **OP-B20** | **OP-P5** | **OP-P9** | **OP-P14** | **OP-P16** | **OP-P17** | **OP-P19** | **OP-P20** |
| **Fold change** | **2** | **2** | **2** | **2** | **2** | **2** | **2** | **2** | **2** | **2** |
| **Total number of bands** | **11** | **13** | **15** | **17** | **19** | **21** | **22** | **24** | **26** | **28** |
| **% of GTS** | **86.42%** | **83.95%** | **81.48%** | **81.48%** | **72.84%** | **72.84%** | **72.84%** | **72.84%** | **72.84%** | **72.84%** |
| **(+): appearance of new bands; (−): disappearance of normal bands.** | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. | **(+)**: appearance of new bands; (−): disappearance of normal bands. |

| Table 10. Effect of MnO2-NPs on electrophoretic of W.S.P. banding patterns of common bean showing the effect of MnO2-NPs in both stages (seedling and flowering). |
|---|---|---|---|---|---|
| **Ser. No.** | **Mw** | **Seedling** | **Flowering** | **Seedling** | **Flowering** |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Primer name** | **OP-A18** | **OP-B18** | **OP-B20** | **OP-P5** | **OP-P9** | **OP-P14** | **OP-P16** | **OP-P17** | **OP-P19** | **OP-P20** |
| **Fold change** | **1260** | **1200** | **1200** | **1280** | **1280** | **1280** | **1280** | **1280** | **1280** | **1280** |
| **Total bands** | **22** | **22** | **22** | **22** | **22** | **22** | **22** | **22** | **22** | **22** |
| **(+): present; (−): absent.** | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. | **(+)**: present; (−): absent. |
2014), this due to the absorption, translocation, and accumulation of nanoparticles in plants, also these particles caught be reacted with plant cells lead to modulate gene expression (Khiew et al., 2011; Salama et al., 2019).

3.7. Toxicity test

The seeds of common beans obtained from the treatment of plants by the various concentrations of MnO2-NPs (0, 10, 20, 30, and 40 ppm) showed nontoxicity by using a Microtox analyzer. Where the effective concentration (EC50) for all samples was ≥100 that indicates they are nontoxic and ecofriendly. Jiang et al. (2012) found that EC50 values for chlorophyll a and phosphate content showed that silver nanoparticles (Ag-NPs) was less toxic than AgNO3.

4. Conclusion

The prerogative of the utilization of manganese in the form of nano is to increment its efficiency to spread through the pores of the plant cell. Moreover, the changes take place in the plant via the nanomaterials enhancing its growth and yield. We can recommend that the manganese nanoparticles can be sprayed on common bean plants at a concentration of 30 ppm to improve the growth, yield, and genetic characteristics, as well as enhanced the quality characteristics of the common bean in terms of its content of proximate analysis, minerals. We propose that more studies will be preferable around the foliar enforcement of manganese nanoparticles with other concentrations of various microelements in the future.

Acknowledgments

The authors are considerable grateful to National Research Centre (NRC) for financially supporting the present study under research project number 12050109.

Disclosure statement

The authors proclaim no conflict of benefit.
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