

Synthesis and applications of MnO₂-enriched urea nanofertilizer on chili *Capsicum annuum L.* plant

B. Ashwini & T. Devasena*

Centre for Nanoscience and Technology, Anna University, Chennai- 600025, Tamil Nadu, India

*E-mail: tdevasenabio@gmail.com

Received 20 September 2024; accepted 8 October 2025

Constant use of several conventional fertilizers on the soil often leads to nutrient losses, soil deterioration, and reduced crop performance. Nanofertilizers are considered one of the most promising alternatives to conventional fertilizers. Plants require both macronutrients NPK (Nitrogen, Phosphorus, and Potassium) and micronutrients (such as manganese, zinc, iron etc.,) for their optimal growth and productivity. Among these, Manganese (Mn) plays a crucial role in several physiological and biochemical processes. Urea is widely used as a fertilizer due to its high nitrogen content (46%), but it can be toxic at higher concentrations. In the present study, we have developed a novel MnO₂-enriched urea nanofertilizer, designed in such a way to provide both nitrogen and manganese in a single conjugate form to overcome Mn deficiency and chlorosis in plants. The nanofertilizer has been synthesized using the hydrothermal method, where KMnO₄ is used as a precursor along with urea (CH₄N₂O) and optimized thoroughly using characterization such as UV-visible absorption, SEM, FTIR, XRD, with varying MnO₂-urea ratios and chosen based on its stable nanoscale features. The effectiveness of the nano fertilizer has been evaluated on *Capsicum annuum L.* (chili) at concentrations of 0, 10, 20, 30 and 40 ppm with a notable improvement in the physical characteristics of plants like root and shoot length, leaf area, and yield was seen around 30 ppm. Chemical characteristics analyses confirmed higher levels of photosynthetic pigments, strong antioxidant capacity, greater mineral accumulation, and improved nutrient use efficiency at this dose. Overall, the MnO₂-enriched urea nanofertilizer functions both as a corrective for chlorosis and as a controlled nutrient source, making it a promising alternative to conventional fertilizers, contributing synergistic nutrient uptake and environmental sustainability.

Keywords: Agriculture, *Capsicum annuum L.*, Manganese, Micronutrient, Nanofertilizer, Urea

Introduction

Capsicum annuum L., or chili, is a widely utilised economic crop that is mostly used as a spice and vegetable all across the world. Due to their medicinal value, they are considered among the easiest plants to cultivate, containing carotenoids used as herbal remedies. They are perennial herbs and shrubs, which can develop over approximately 80–120 days¹. January - March was thought to be the most suitable time for planting chilli plants² (summer plants). It is a sun-loving plant, so the water required is less compared to other plants. Nutrient deficiencies cause many problems for chili (*Capsicum annuum L.*) plants. Some of the common deficiencies that are seen in chili are nitrogen deficiency, where the leaves turn yellow or pale green, and the plant may have stunted growth. This is considered one of the major problems faced by the chili plant, and with the right amount of nitrogen as fertilizer³, this deficiency can be avoided. Another main problem faced in chili plants is Mn deficiency because of low manganese level in soil, where the

plants undergo chlorosis i.e., yellowing of leaves and pale brown spots will appear around the interveinal regions of leaves^{4,5} which leads to drying of plants.

MnO₂-enriched urea nanofertilizers are an innovative approach to provide nitrogen because of urea and manganese due to MnO₂ nanoparticles (NPs) present in the conjugate to help the plants. The nano fertilizers consist of urea (CO(NH₂)₂) particles that are combined with MnO₂ NPs. NPs present in this conjugate increase the surface area of the fertilizer particles, which enhances their interaction with plant roots, leading to increased nutrient uptake^{6,7}. The use of MnO₂-enriched urea nanofertilizers can offer several benefits, including improved plant growth, increased yield, and better nutrient use efficiency. Additionally, the use of nanofertilizers will reduce the amount of fertilizer needed to achieve the same results, which can decrease the environmental impact of agriculture⁸⁻¹⁰. The MnO₂-urea nanofertilizer minimizes nutrient losses, lowers costs, and reduces fertilizer input while improving plant growth and stress resilience. By

reducing the risks of eutrophication and greenhouse gas emissions, its controlled release mechanism promotes ecologically friendly practices. Higher and more consistent crop yields can result from improved germination, plant vigour, and stress resistance, which directly boosts farmers "productivity and profitability". These findings demonstrate the practical and societal value of the nanoconjugate for sustainable agriculture.

Manganese is an essential micronutrient that is required by plants for various physiological processes, including photosynthesis, enzyme activation, maintaining strong growth and development, and nitrogen metabolism^{1,11}. Inadequate manganese can result in stunted growth, reduced yield, and poor-quality crops. Nanofertilizers are a relatively new type of fertilizer that has been developed by incorporating NPs into conventional fertilizers. These NPs can improve the efficiency of nutrient uptake by plants, reduce fertilizer loss, and enhance plant growth¹². Mn deficiency is one of the serious, widespread plant nutritional disorders seen in almost all types of soil, including dried, well-aerated, and calcium-rich soils and soil containing high amounts of organic matter.

Urea is widely used as a fertilizer due to its high nitrogen content (46%), but it can be toxic at higher concentrations¹³. To reduce the usage of urea, we can go for nano-sized urea particles, where the usage will be less compared to the bulk urea particles. Constant use of chemical fertilizers like these on land can lead to the loss of certain nutrients and increase the toxicity level of soil and plants¹⁴. To overcome toxicity, the use of bulk fertilizer has to be reduced, and to avoid chlorosis due to manganese deficiency and nitrogen deficiency in plants, to increase the photosynthetic reaction, improve anti-oxidant property, and for better growth & development of *Capsicum annuum L.* chili plant, the required quantity of the synthesized MnO₂-enriched urea nano fertilizers is used.

However, the use of nanofertilizers is still a developing area of research, and there are concerns about the potential long-term effects of NPs on the environment and human health¹⁵. Therefore, it is important to continue studying the safety and effectiveness of nanofertilizers before they are widely adopted.

Perfileva *et al.*¹⁶ investigated Mn-NPs synthesis, mechanisms of influence on plant resistance to stress, and prospects for application in agricultural chemistry. The protective effect of Mn NPs associated with their antioxidant activity, activation of systemic acquired

resistance (SAR), and pronounced antimicrobial activity against phytopathogens are discussed. Salama *et al.*¹⁷ investigated the effects of MnO₂-NPs on plants through foliar application at varying concentrations. 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. The utilization of Mn in the form of nano increases its efficiency, and at a concentration of 30 ppm, the growth, yield, and genetic characteristics are improved, as well as enhancing the quality characteristics in terms of its content of proximate analysis and minerals in the plant. Samssoon *et al.*⁶ studied the MnO₂ NPs as a nano fertilizer and its impact on growth, photosynthetic pigment, and non-enzymatic antioxidant of *Vigna unguiculata* cultivar. The yield and productivity were checked by soil and foliar application of MnO₂ NPs. The effect of soil application was better compared to foliar application as it enhances the chlorophyll, growth characteristics, and non-antioxidant enzymes of the plant. Sunil *et al.*¹⁸ has performed comparative study of nano nitrogen and nano zinc nutrition on growth, yield, and profitability of chilli plant. They investigated its growth and yield responses to foliar applications of nano nitrogen and zinc alongside conventional urea and ZnSO₄ which shows improved growth and yield attributes & green chili yield and shows utilization enhancement in plant. Many groups have focussed on use of either MnO₂ or nano-urea as nanofertilizer individually but in the present study, we used conjugate of MnO₂ and urea NP, which fulfils the requirement of both MnO₂ and urea as compared to commercially available nano fertilizers and other bulk fertilizers.

Experimental Section

Materials

Potassium permanganate (KMnO₄) and urea (extra pure, AR- 99.5%) (CH₄N₂O) were purchased from SRL. All the chemicals used in this study were of analytical grade. All the solutions for characterizations were made using deionized (DI) water and the studies were carried out at ambient temperature.

Methodology

The synthesis of MnO₂-enriched urea nanofertilizer involves several steps, the first of which is the preparation of a precursor for MnO₂-enriched urea NPs. The hydrothermal method was used as the synthesis process because of its uniform formation of

NPs under high vapour pressure from a high-temperature aqueous solution. Thus, the property of the product is improved.

Preparation of precursor

0.01 M KMnO₄ solution was prepared by taking 1g KMnO₄ in a container and mixing it with 35 mL of distilled water which acts as the precursor. Urea (0.5 g) was taken and mixed with 15 mL distilled water, which forms a colourless solution. The solution was made to 50 mL followed by further processing.

Synthesis of MnO₂-enriched urea NPs

The prepared solutions were mixed together by constant stirring with the help of a magnetic stirrer for about 30 min, and the pH was maintained between 0.5 - 1. After they are completely mixed with each other, they are transferred to a teflon-lined autoclave and are tightly sealed. It was kept at 100°C for 12 h. After that, the autoclave was cooled to room temperature and the pressure was released. A black-brown precipitate was formed and was separated by using the Whatman filter paper and washed with distilled water/ ethanol twice or thrice to remove impurities.

The washed pellet was collected and dried under vacuum @70°C for 5-6 h. Then the dried sample was ground using a mortar and pestle for the uniform formation of NPs. Thus, the MnO₂-enriched urea nanofertilizer was finally prepared and was stored tightly. The colour change of dark violet to black-brown precipitate during the synthesis of nano fertilizer suggests the formation of MnO₂-enriched urea NPs. With the help of the same synthesis process, different ratios of this nanofertilizer are prepared and checked for better formation of NPs. The better-formed ratio was analysed and used for further plant application.

Plant preparation

The plant used for the application is *Capsicum annuum L.* chili plant. The plants are grown from day 1 for about 3 weeks and are planted separately for the use of MnO₂-enriched urea nano fertilizer onto the soil. The plant's vegetative parameters such as root length, shoot length, leaf length and the chemical characteristics of *Capsicum annuum L.* plant were investigated.

Transplanting seedling

Once the small plants show true leaves, they were transplanted or repotted to a larger pot (10 inches), to provide more space for the seedlings to grow and

develop. One plant per pot was planted. Red soil was used along with cocopeat as the soil mixture. Then the plants were labelled and checked regularly for their pH, moisture, and light using analytical instrument.

- pH level: Maintained around ~ 7 (neutral) and checked using a pH meter.
- Moisture level: Maintained around ~4-5 (intermediate level) with the help of a moisture meter.
- Light: the chili plant requires high sunlight. A photometer (light meter) was used near the plant to maintain a light range of ~700, which helps to increase photosynthesis.

Application of MnO₂-enriched urea nano fertilizer on soil

For application, 3:1 MnO₂-urea ratio was selected based on the physicochemical characterization results such as UV-visible analysis, where a distinct absorption band at ~293 nm, confirming strong MnO₂-urea interaction; FTIR spectra revealed clear shifts in the urea C=O and N-H bands, indicating successful conjugation with MnO₂, low Mn ratios (1:3, 1:1) showed weaker or less pronounced shifts with weaker interaction; XRD patterns exhibited sharp diffraction peaks, indicating high crystallinity and phase purity in the 3:1 formulation, whereas lower Mn ratios displayed broader and less intense peaks; SEM images further demonstrated well-dispersed NPs with uniform morphology in 3:1. These results collectively validated that the 3:1 ratio possessed the most stable structure and highest potential for nutrient delivery. It was therefore chosen for further plant application studies. So, the prepared ratio (3:1) was used. A range of 10–40 ppm interval was chosen because it represents agronomically relevant and environmentally safe doses commonly reported for nano-fertilizer and MnO₂ nanoparticle applications. Salama et al.¹⁹ showed that MnO₂-NPs at 10–30 ppm improved bean growth, while a recent review (Ayenew et al.²⁰ & Haydar et al.²¹) highlights 10–40 ppm as a practical and safe window for foliar/soil application of nanofertilizers. Testing at 10, 20, 30, and 40 ppm allows us to map the dose-response relationship and identify the optimum concentration for growth enhancement. The varying concentrations (i.e., 10 ppm = 10 mg/L) are mixed with water to make 1 L. Then, the prepared solution was poured onto the soil, around 250 mL per pot. The vegetative parameters, such as root growth, shoot

length and leaf length were observed after 15-20 days of application at a regular interval of time.

Preparation of plant extract

The leaves were separated from the plant material, washed, and dried at room temperature. Once dried, the leaves were ground to a fine powder with the help of a mortar and pestle. For extraction, 20 g of plant sample was weighed and 20 mL of acetone was added to make it grind smoothly. Once done, they were centrifuged at 5000 rpm for 10 min and were filtered and separated. The resulting extract was stored for further analysis.

Preparation of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

A solution of the radical is prepared by dissolving 2.4 mg 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 100 mL methanol. A plant extract solution (5 μ L) was added to 4 mL of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 60 min in the dark. Absorbance of the reaction mixture was measured spectrophotometrically. Thus, the antioxidant property of the plant is measured using this DPPH Assay.

Characterization of NPs

The NPs were characterized by using UV-visible spectrophotometer (T90+ UV/vis spectrophotometer) to obtain the absorption or reflectance of NPs; Scanning Electron Microscopy SEM (FEI Quanta FEG 200-High Resolution Scanning Electron Microscope) for studies of morphology, topography, contrast and image formation of NPs; Fourier transform infrared FTIR (JASCO FTIR-6600 spectrometer) to determine the functional groups present in the compound and X-ray diffraction XRD (PANalytical, Netherlands) are used to analyse the lattice parameter and crystalline size of the powder sample for analysis of the NPs present.

Results and Discussion

Synthesis and preliminary visual Confirmation

Hydrothermal synthesis was used for the synthesis because it enabled a controlled growth of uniform NPs and also improves the crystallinity. Mild aqueous conditions (100-120°C) help in preserving urea from thermal degradation. Unlike other techniques requiring high calcination or harsh chemicals, this technique provides intimate MnO₂-urea conjugation, essential for stable nanofertilizer formation and

efficient nutrient delivery. Acidic pH was maintained during synthesis for the reduction of KMnO₄ to MnO₂, which occurs preferentially under acidic conditions, yielding pure MnO₂ nanostructures with controlled nucleation whereas in neutral or alkaline media, side reactions lead to mixed manganese oxides or amorphous precipitates. Moreover, acidic conditions stabilize Mn⁴⁺ and prevent urea hydrolysis, thereby ensuring successful conjugation of MnO₂ with urea. Fig. S1 (Supplementary Information) shows the synthesis of MnO₂-enriched urea nanofertilizer after the synthesis process under ambient temperature. The black-brown precipitate was formed finally, and it confirmed the formation of MnO₂-urea nanofertilizer formation.

Physicochemical characterization

The UV-visible spectra (Fig. 1) were recorded for different ratios of MnO₂ and urea (1:3, 3:1, 1:1). Among the varying ratios the 3:1 sample showed a better absorption and a sharp peak around ~293 nm, which confirms the presence of the MnO₂-urea NPs. The absorption band observed at ~293 nm is consistent with MnO₂ NPs, which typically show charge transfer transitions between Mn⁴⁺ and O²⁻ in the 280–310 nm range^{7,19}.

SEM analysis provided the surface morphology of MnO₂-enriched urea NPs at different ratios (i.e., @ 1:3, 1:1, and 3:1) at different magnifications, which is displayed in Fig. 2. SEM images show the presence of nano-sized irregular arrangement of spindle fibre-like NPs, which are observed and confirmed the formation of MnO₂-enriched urea NPs of concentration 1:0, 0:1, 1:3, 1:1 and 3:1 respectively. The better-formed

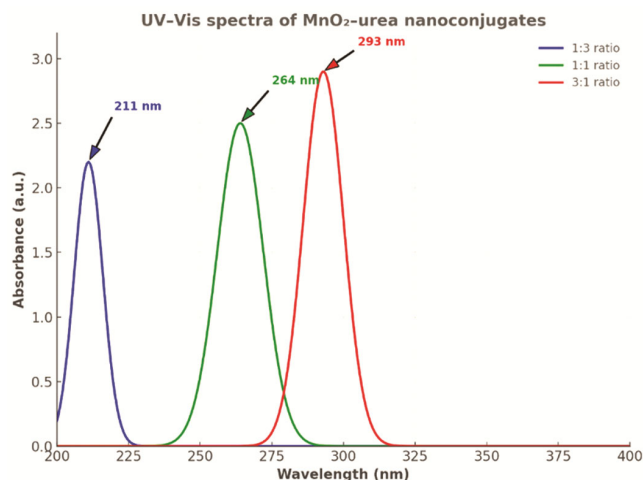


Fig. 1 — UV-visible spectra of MnO₂-urea nanoparticles

MnO₂-urea NPs are seen in a ratio of 3:1 with an average size of around 90 nm and the morphology was much better compared to other ratios.

X-ray diffractograms of MnO₂-enriched urea NPs for the better formed ratio 3:1 is shown in Fig. 3c. It shows intense peaks, obtained at $2\theta = 12.4, 21.6, 24.7, 31.2, 37.7, 40.5, 47.3, 54.3, 57.1$ and 65.7 corresponding to the (110), (200), (220), (310), (211), (301), (411), (600), (521) and (002) plane of MnO₂ and Urea, respectively. The results confirmed the crystallization of phase-pure MnO₂ NPs with a well-defined crystalline morphology (JCPDS 14-644). X-ray diffractograms for ratios MnO₂ control (3:0) and urea control (0:3) are taken and the peaks for MnO₂ control were obtained at $2\theta = 12.6, 18.1, 28.6, 37.5, 41.8, 49.7, 55.8, 60.05,$ and 69.2 which are assigned to the (110), (200), (310), (211), (301), (411), (600), (521), (002) and (741) plane of MnO₂,

respectively, which correspond to the crystallization of phase-pure MnO₂ NPs in crystal morphology. The peaks for urea control are obtained at $2\theta = 11.3, 18.8, 21.9, 23.6, 27.7,$ and $37.1, 38.3$ and 45.7 , which are assigned to the (020), (210), (210), (002), (121), (131), (141) and (322) plane of urea, respectively.

FT-IR spectra of MnO₂-urea NPs for different ratios 3:0, 0:3, 1:1, 3:1, and 1:3 are presented in Fig. 4. The band located around 570 cm^{-1} describes the Mn-O vibrations due to MnO₂ NPs. A broad peak in the range $3400\text{-}3000\text{ cm}^{-1}$ is attributed to the symmetrical stretching vibrations of Mn-O and O-H bending vibrations of the hydroxyl group. The NH stretching of amides (at 3187 cm^{-1}) is formed due to the presence of the amine group because of the use of urea (CH₄N₂O) and the C=O stretching at 1646 cm^{-1} because of the formation of the carboxyl groups.

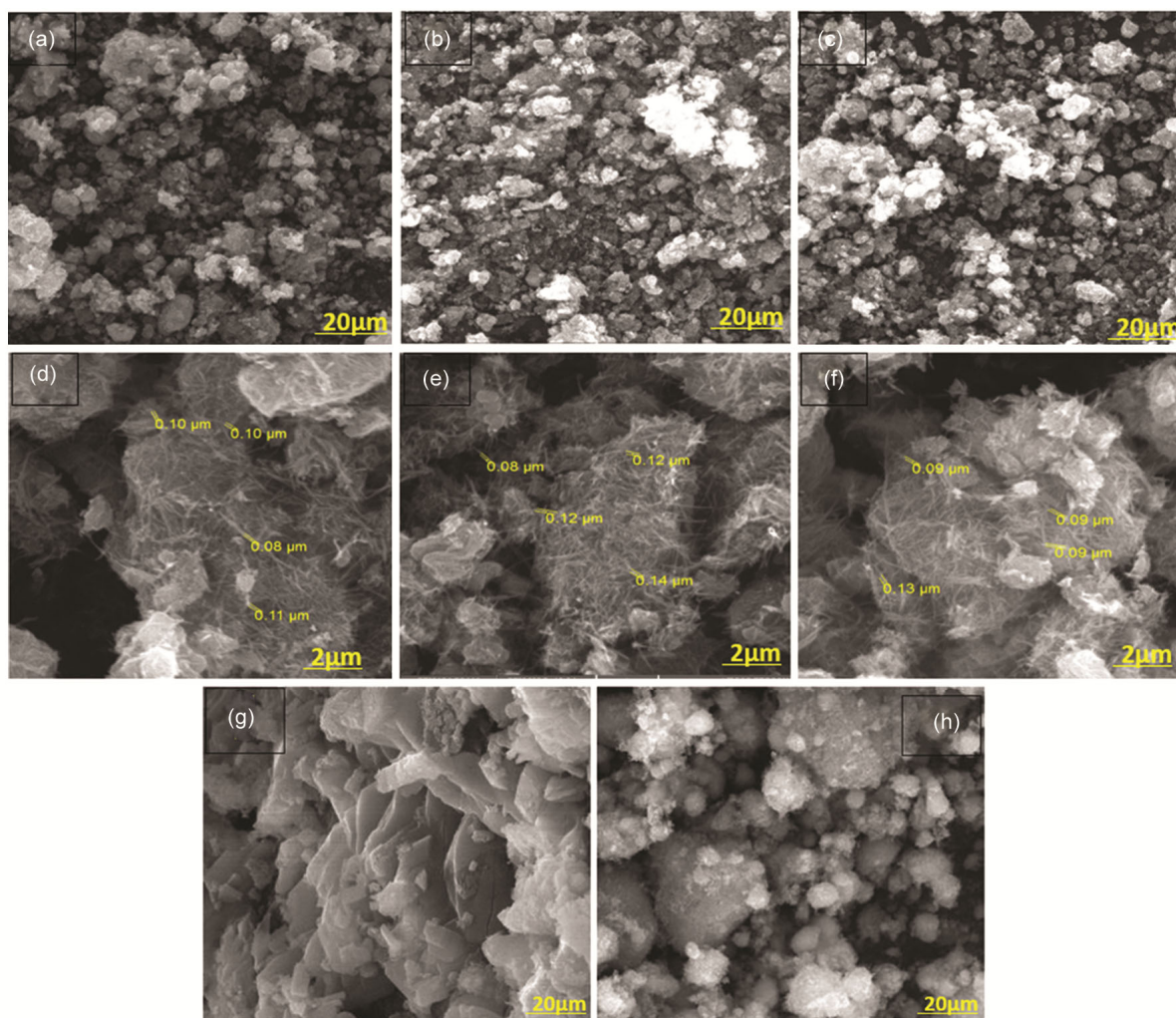


Fig. 2 — SEM micrographs of (a) 1:3, (b) 1:1, (c) 3:1, (d) 1:3, (e) 1:1, (f) 3:1, (g) 3:0 and (h) 0:3 MnO₂-urea NPs

Effect of nanofertilizer on chili plant

Vegetative growth characteristics

Germination percentage (GP) estimates the viability of a population of seeds and was calculated using the following formula.

$$GP = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

The germination rate provides a measure of the time course of seed germination and in MnO_2 -enriched urea nanofertilizer, the growth percentage was around 97%, 30% for urea control, and 20% for normal control seeds & MnO_2 control. Fig. S2 (a-d) (Supplementary Information) represents the germination of seeds where (a) is the control (0:0), (b) is germination of seeds at (3:0) control, (c) is the germination of seeds at (0:3) control and (d) is the germination of seeds using the MnO_2 -enriched urea nano fertilizer in the ratio (3:1). Table.1 represents the germination percentage of seeds.

Physical studies

Plant growth: (Root and shoot length)

The plant growth after day 120 days with different ratio & concentration of nanofertilizer is shown in Fig. S3. As evident from Table 2, MnO_2 -enriched urea nanofertilizer (3:1) showed better plant growth when compared to control (0:0), (3:0) & (0:3). It shows that around 30 and 40 ppm, the plant shoot length was drastically increased after day 120. The root length was also increased during day 120 when compared to control measures.

Number of leaves & surface area of leaves

The number of leaves in plants indicates a plant's physiological age and leaf surface area refers to the total area of all the leaves on a plant. The large surface area of the plant helps it to absorb as much sunlight as possible which is required for the plant through the process of photosynthesis. Therefore, surface area A =

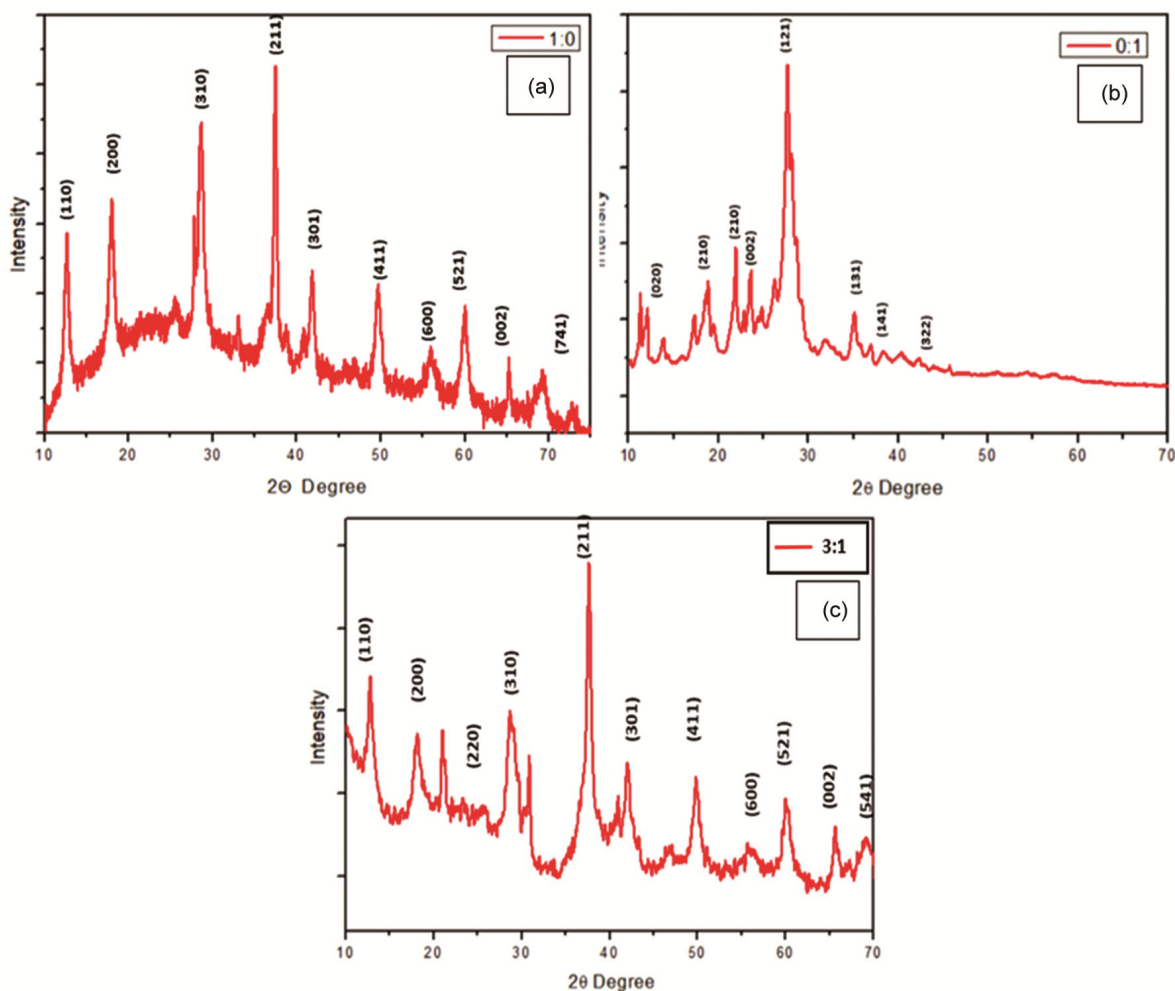


Fig. 3 — X-ray diffractograms of MnO_2 -urea NPs

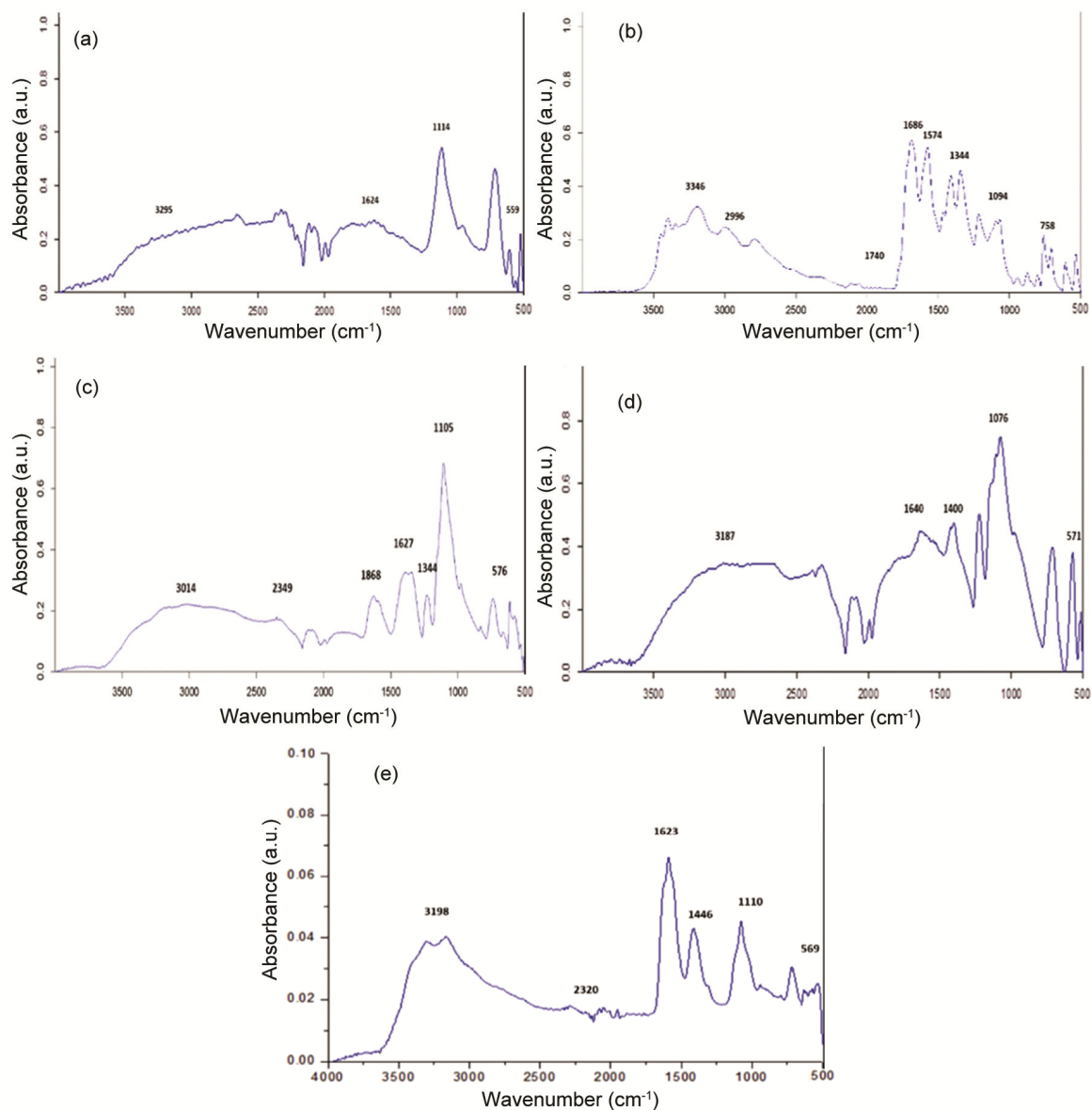


Fig. 4 — FTIR spectra of synthesized MnO₂- Urea NPs where (a) 3:0, (b) 0:3, (c) 1:1, (d)3:1 and (e) 1:3

Table 1 — Germination percentage of chili plant after nanofertilizer application at different ratios

SI No.	D1	D2	D3	D4	D5	D6	D7	Sum germinated	GP (%) Germination percentage	MGT (Mean germination time) (Days)
Control 0:0	0	0	0	0	0	2	7	9	19.44	0.778
MnO ₂ -Control 3:0	0	0	0	0	2	5	6	13	20.00	0.538
Urea control 0:3	0	0	0	2	4	8	9	23	30.00	0.304
MnO ₂ -Urea NF 3:1	0	0	3	7	19	24	35	88	97.22	0.080

cLW , where c is called the Montgomery parameter (0.68). The Montgomery equation (ME) assumes that leaf area (A) is a proportional function of the product of leaf length (L) and width (W). The calibration of the leaf is done by the length and width as shown in Fig. 5 and Table 3 represent the surface area of the chili leaf.

Nature of leaves

The visible changes occur in plants after the application of MnO₂-enriched urea nano fertilizer on *Capsicum annum L.* plant. The changes in size of leaves are seen for different ratios which are shown in Fig. 6 and the sizes are discussed in Table 3.

Table 2 — Plant growth (root and shoot length)

SI No.	Control						MnO ₂ -urea nanofertilizer (3:1)							
	Shoot length (cm)			Root length (cm)			Shoot length (cm)				Root length (cm)			
	0:0	3:0	0:3	0:0	3:0	0:3	10	20	30	40	10	20	30	40
DAY 45 (TRANSPLANT)	11.3 ±1.18	11.3 ±1.18	11.3 ±1.18	2.2 ±0.20	2.2 ±0.20	2.2 ±0.20	10.6 ±0.42	10.6 ±0.42	10.65 ±0.42	10.64 ±0.42	2.5 ±0.19	2.5 ±0.04	2.5± 0.07	2.5 ±0.09
DAY 70	14 ±0.77	25 ±2.32	22.67 ±1.9	4.2 ±0.34	5.54 ±0.11	4.86 ±0.36	34 ±1.34	35.7 ±1.1	42 ±0.7	45 ±0.7	6.74 ±0.1	7.1 ±0.07	7.2 ±0.04	7.3 ±0.04
DAY 90	47.3 ±1.95	61.67 ±1.61	56.34 ±1.18	6.5 ±0.07	6.54 ±0.14	6.24 ±0.11	65 ±0.77	71 ±1.3	81 ±0.7	84 ±0.7	7.3 ±0.11	7.3 ±0.09	7.5 ±0.1	7.6 ±0.1
DAY 120	61.8 ±0.80	62.67 ±1.53	57.17 ±1.04	7.2 ±0.1	6.54 ±0.41	6.94 ±0.15	102.4 ±5.16	115. 6±2.9	136 ±4.02	140 ±3.87	7.5 ±0.09	7.5 ±0.07	8 ±0.2	8.2 ±0.2

Table 3 — Calculated surface area of leaves

SI. No.	Leaf surface		Surface area	Total number of leaves
	Length (L) (cm)	Width (W) (cm)	A = cLW	
Control 0:0	5.08 ±0.06	3.07 ±0.08	10.60	92.34 ±0.14
MnO ₂ Control 3:0	4.56 ±0.18	2.9 ±0.3	8.99	83.1667 ±2.2
Urea Control 0:3	5.06 ±0.2	3.26 ±0.3	11.22	95.5 ±1.6
10ppm	5.94 ±0.29	3.24 ±0.19	13.08	167 ±2.05
20ppm	6.4 ±0.16	4.17 ±0.11	18.14	164 ±2.44
30ppm	7.0 ±0.54	4.44 ±0.08	21.13	202 ±2.44
40ppm	7.2 ±0.35	4.34 ±0.04	21.24	127.34 ±2.36

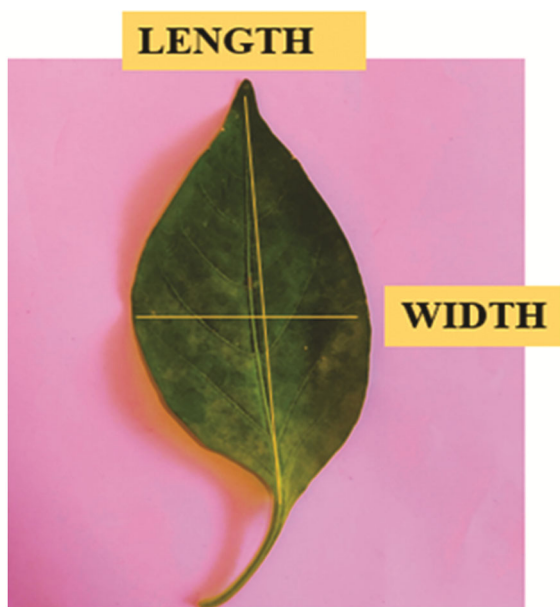


Fig. 5 — Calibration of leaf length and width

Yellowing in leaves is seen at 40 ppm and any other changes in plants were noted.

Flowering and fruiting

The fruiting and flowering observations are made after 120 days and are shown in Fig. S4. Table 4 shows the fruiting and flowering of chili at 0, 10, 20, 30 and 40 ppm. In 30 ppm concentration, the flowering and fruiting are better when compared to other concentrations.

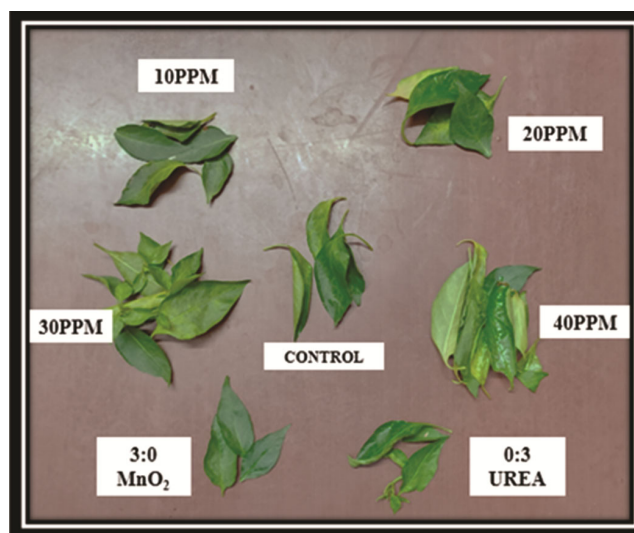


Fig. 6 — Nature of leaves

Chemical characteristics

Photosynthetic pigments

The fresh leaves were collected after 120 days of fertilizer application to extract photosynthetic pigments using 85% acetone. The absorbance of chlorophyll a (Chl. a), chlorophyll b (Chl. b), and carotenoid (Cart.) were measured by UV-visible spectrophotometer at 430, 453 and 660 nm, respectively, where peaks of chlorophyll a will be seen at 430 nm, chlorophyll b at 660 nm and carotenoid pigment peak at 453 nm are shown in Fig. 7.

Minerals determination

The minerals present in soil are determined by using soil testing from Soil Testing Laboratory, Kanchipuram. The concentration of nitrogen and Mn content changes for different ppm concentration of nanofertilizer to the applied soil is represented in Table 5. The minerals concentration was increased at 30 ppm and 40 ppm but at high concentration i.e., 40 ppm, the acidic content (pH) of soil is increased which leads to loss of leaves due to yellowing and drying. So, it is advisable to go for 30 ppm for better growth of the chili plant.

Antioxidant property using DPPH radical scavenging assay

A solution of the radical assay is prepared by dissolving DPPH in methanol (CH₃OH), which is considered as the stock solution. The plant extract solution was added to DPPH assay. The solution was centrifuged and kept at room temperature for 60 min in the dark. Absorbance of the reaction mixture was measured spectrophotometrically by UV-visible spectroscopy. Absorbance of the DPPH radical was observed at 515 nm, for both with and without plant extract solution (Fig. 8). The blank solution of DPPH was also measured for calibration. The increased antioxidant property is seen in 30 ppm which is shown clearly in Fig. 8 and the absorbance is represented in Table 6. The capability to scavenge the DPPH radical was calculated using the following equation.

$$DPPH\ Scavenged\ effect\ (\%) = ((AA - AB)/AA) \times 100$$

Table 4 — Fruiting and flowering observation

SI No.	No. of flowering	No. of fruiting (yield)
Control	5.4 ±0.45	2 ±0.78
MnO ₂ Control 3:0	12.8 ±0.76	3.67 ±0.58
Urea Control 0:3	10.16±1.25	4.34 ±0.58
10 ppm	14.67 ±0.44	4.67 ±0.44
20 ppm	16.34 ±0.94	10.34 ±0.44
30 ppm	25.34 ±1.18	16 ±0.77
40 ppm	11.67 ±1.18	4.67 ±1.18

Table 5 — Minerals present in soil after the use of nanofertilizer

SI No.	Macro nutrients concentration			Micro nutrient concentration	Electrical conductivity	Potential of hydrogen
	N (kg/ha)	P (kg/ha)	K (kg/ha)	Mn (ppm)	EC(dS/m)	pH
Control	79.6	48	366.3	5.2	0.25	7.1
MnO ₂ 3:0	80	32	230.3	6.3	0.22	7.1
Urea 0:3	90.5	33	223.6	5.3	0.21	7.1
10 ppm	88.8	30	219.6	5.3	0.19	7.2
20 ppm	94.8	30	199.6	6	0.16	7.2
30 ppm	95.2	35.8	204.6	6.4	0.12	7.3
40 ppm	80.4	38.3	249.6	6.9	0.10	8.2

Where, AB is the absorbance of the blank at t= 0 min and AA is the absorbance of the antioxidant at t= 60 min.

Nutrient use efficiency (NUE)

Nutrient use efficiency (NUE) is a critically important concept in the evaluation of crop production systems. It is used as a short-term

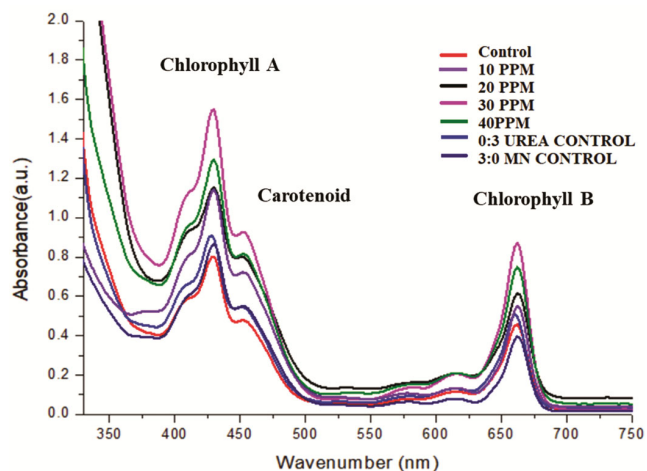


Fig. 7 — Absorbance of photosynthetic pigments

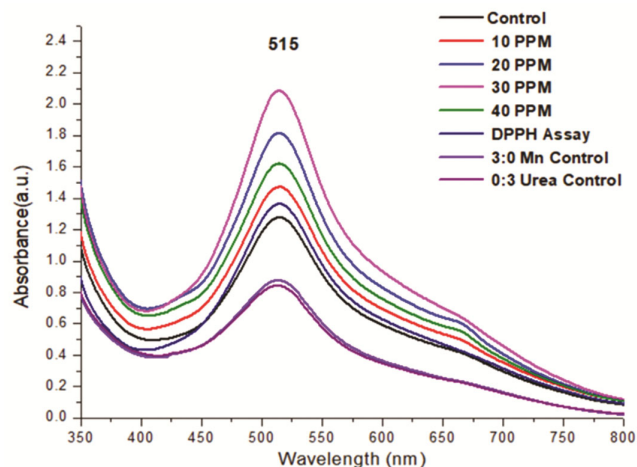


Fig. 8 — Absorbance of antioxidant property using DPPH radical scavenging assay

indicator of the impact of applied nutrients on productivity. The following formula was used to measure NUE, which is the return in the chilli yield per unit of fertilizer nutrient applied. The NUE is calculated and shown in Table 7 from which we can clearly note the increased nutrient use efficiency of about 1.19 ppm in 30 ppm concentration compared to other varying concentrations.

$$NUE \text{ (kg/ha)} = \text{Yield (kg/ha)} / \text{Quantity of Fertilizer (kg/ha)}$$

Mechanism of action of nanofertilizer in chili plants

The MnO₂-urea conjugate works as a "smart" nano fertilizer, providing a dual benefit of sustained nutrient delivery and stress mitigation. This mechanism of nutrient uptake is shown in Fig. 9. The primary function of the nanofertilizer is the controlled release of N and Mn into the soil, ensuring their efficient uptake by the chili plant. Upon nanofertilizer application, soil moisture hydrates the nanocomposite and the urea compound (CO(NH₂)₂) will undergo gradual enzymatic hydrolysis catalysed by the soil urease. Thus, slowly ammonium ions (NH₄⁺) are released, preventing the rapid surge of nitrogen, unlike other conventional urea fertilizers. This process will decrease nitrogen loss through leaching and volatilization.



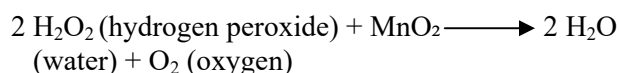
Simultaneously, in the rhizosphere, the root exudates of chili plant (e.g., organic acids and other

microbes), therefore, stimulate microbial activity, reduce the MnO₂ NPs to the more bioavailable Mn₂⁺ form. This slow, in-situ conversion provides a steady supply of a critical micronutrient as follows.



The chili plant roots absorb the released NH₄⁺ and Mn₂⁺ via specific transporters. The consistent supply ensures that the plant has the necessary building blocks for essential processes like chlorophyll synthesis (photosynthesis) and protein formation, leading to improved growth and yield.

The MnO₂ component also serves as a potent antioxidant, preventing the chili plant from oxidative damage caused by various stress. Abiotic stresses (such as drought, salinity, and heat) and biotic stresses (e.g., pathogen attacks) cause the plant to produce a burst of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂). Excess ROS can lead to oxidative stress, damaging cells and inhibiting growth. They catalytically decompose excess H₂O₂ into water and oxygen as per the follow equation, mimicking the function of natural plant enzymes like catalase and peroxidase. This modulation of ROS levels prevents oxidative damage while maintaining the low levels of ROS needed for cellular signaling, thereby enhancing the chili plant's resilience.



Environmental safety and toxicity of nanofertilizer

Soil mineral analysis after application of MnO₂-urea nanofertilizer (10–40 ppm) confirmed that Mn concentrations remained well within the agronomically safe range. Reported background Mn in agricultural soils is typically 20–300 ppm, while toxicity is generally observed only above 300–500 ppm depending on soil pH and crop species (FAO soil fertility guidelines)^{22,23}. In our study, the highest soil Mn content recorded was 6.9 ppm at 40 ppm application (Table 6), far below these toxicity

Table 6 — Antioxidant property % using DPPH Assay

SI NO	Antioxidant absorbance(nm)	DPPH scavenged effect (%)
DPPH	1.227	NIL
Control	1.355	10.43
MnO ₂ 3:0	0.882	28.11
Urea 0:3	0.844	31.21
10 ppm	1.462	19.15
20 ppm	1.824	48.65
30 ppm	2.077	69.27
40 ppm	1.621	32.11

Table 7 — Nutrient use efficiency (NUE)

SI No.	Quantity of Fertilizer (kg/ha) in ppm	Yield (no. of chilli's) (After 120 days)	Nutrient use Efficiency (kg/ha) NUE	Total nutrient uptake NUE in ppm
Control	0	2 ±0.78	0	0
10 ppm	10	4.67 ±0.44	0.46	1.04
20 ppm	20	10.34 ±0.44	0.51	1.15
30 ppm	30	16 ±0.77	0.54	1.19
40 ppm	40	4.67 ±1.18	0.35	0.78
MnO ₂ 3:0	30	3.67 ±0.58	0.36	1.03
Urea 0:3	30	4.34 ±0.58	0.43	1.04

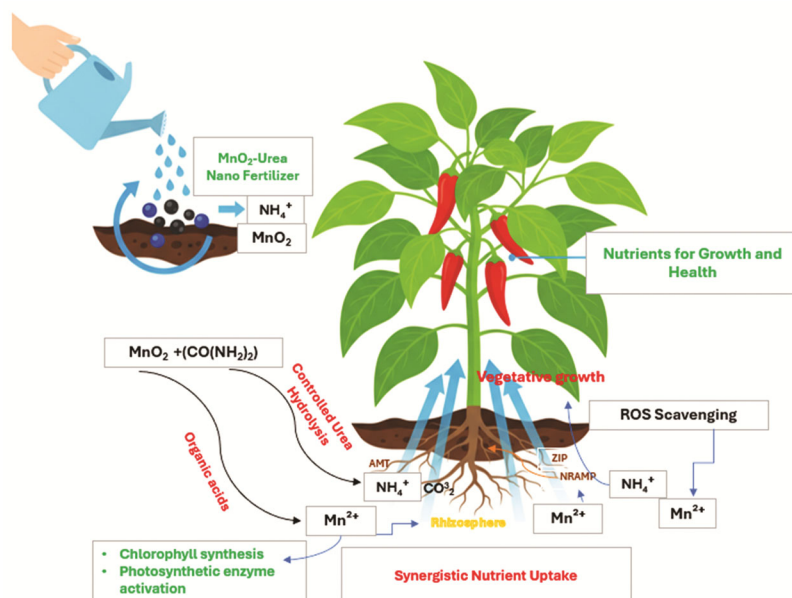


Fig. 9 — Mechanism of action of the MnO₂- enriched urea nano fertilizer

thresholds. This indicates that the tested concentrations are unlikely to pose Mn toxicity risks to chili plants or to soil ecology under the experimental conditions. Moreover, the conjugated MnO₂-urea formulation facilitates gradual nutrient release, reducing the chance of sudden Mn spikes or nutrient imbalance compared with bulk fertilizers. Nevertheless, long-term studies under field conditions and repeated application cycles will be necessary to confirm the absence of cumulative effects on soil health and microbial activity.

Future perspective

Beyond correcting the nutrient deficiencies, MnO₂-urea nanofertilizer may even influence plant responses to biotic and abiotic stress. Mn plays a central role in the water-splitting reaction of photosystem II and in antioxidant enzyme activation; thus, Mn supplementation has been shown to improve tolerance to drought, salinity, and oxidative stress^{16,18}. The conjugated nanoformulation employed in this work further enhanced the antioxidant capacity of chili plant (69.2% DPPH scavenging at 30 ppm), which may help minimize stress caused by ROS. In addition, Mn NPs have been shown to decrease pathogen load and improve systemic acquired resistance¹⁶, suggesting that they may be a potential biotic stress protector. Although these effects were not the main focus of this work, future research ought to look at the potential benefits of MnO₂-urea nanofertilizer for enhancing stress resistance in the face of pathogen, salinity, and drought challenges.

Conclusion

MnO₂-enriched urea nanofertilizer was successfully developed containing macronutrient (N) and micronutrient (Mn) and delivered to the *Capsicum annuum L.* plant rhizosphere in a regulated manner. Among different ratios of nanofertilizers, (3:1) was chosen and among varying concentrations 30 ppm shows good physical and chemical characteristics. Above 30 ppm, yellowing and falling of leaves resulted due to high Mn content which cause changes in soil pH to acidic in nature which was seen around 40 ppm, thus disrupting photosynthesis and enzyme activity in the plant. Therefore, this study has concluded that, MnO₂-enriched urea nanofertilizer leads to better growth of *Capsicum annuum L.* plant at 30 ppm and helps to increase the photosynthetic pigments in plants to avoid chlorosis caused due to Mn deficiency and the nitrogen content of the plant is increased by using urea alongside. Thus, MnO₂-urea acts as a smart, multifunctional nanofertilizer that ensures controlled nutrient release to meet the chili plant's nutritional needs efficiently and provides stress mitigation by scavenging harmful ROS. This dual action makes it a superior and sustainable alternative to conventional fertilizers.

Conflict of interest

The authors declare no conflict of interest.

Supplementary Information

Supplementary information is available on the website <http://nopr.niscpr.res.in/handle/123456789>.

References

- Badia A D, Spina A A & Vassalotti G, *Capsicum annum L.*: An overview of biological activities and potential nutraceutical properties in humans and animals, *J Nutr Ecol Food Res*, 4 (2017) 167.
- Das S & Sharangi A B, Impact of climate change on spice crops, *Indian Spices*, (2018) 379.
- Sunil C, Kadam P V, Kanavi G B J, Onte S, Salimath S B, Jeevan H R, Mallikarjuna H B, M, Chandra M S & Seema N U, Comparative assessment of nano nitrogen and nano zinc nutrition on growth, yield and profitability of chilli (*Capsicum annum. L.*), *J Plant Nutr*, 47 (2024) 916.
- Jeyakumar, P. & Balamohan, T. Micronutrients for horticultural crops. Training manual on role of balanced fertilization for horticultural crops, *TNAU,Coimbatore-03*. (2007).
- Massimi M & Radocz L, The action of nutrients deficiency on growth biometrics, physiological traits, production indicators, and disease development in pepper (*Capsicum annum L.*) plant: A review, *Am Eurasian J Sustain Agric*, 15 (2021) 1.
- Samsoon S, Azam M, Khan A, Ashraf M, Bhatti H N, Alshawwa S Z & Iqbal M, Green-synthesized MnO₂ nanofertilizer impact on growth, photosynthetic pigment, and non-enzymatic antioxidant of *Vigna unguiculata* cultivar, *Biomass Convers Biorefin*, 14 (2022) 26943.
- Zhao W, Ma T, Zhou P, Wu Z, Tan Z & Rui Y, Insights into the effect of manganese-based nanomaterials on the distribution trait and nutrition of radish (*Raphanus sativus L.*), *Plant Physiol Biochem*, 207 (2024) 108428.
- Channab B E, Idrissi A E, Ammar A, Dardari O, Marrane S E, El-Gharrak A, Akil A, Essemli Y & Zahouily M, Recent advances in nano-fertilizers: Synthesis, crop yield impact, and economic analysis, *Nanoscale*, 16 (2024) 4484.
- Srivastav A L, Patel N, Rani L, Kumar P, Dutt I, Maddodi B S & Chaudhary V K, Sustainable options for fertilizer management in agriculture to prevent water contamination: A review, *Environ Dev Sustain*, 26 (2024) 8303.
- Meena D S, Gautam C, Patidar O P, Meena H M & Prakash V G, Nano-fertilizers is a new way to increase nutrients use efficiency in crop production, *Int J Agric Sci*, 9 (2017) 3831.
- Tripathi D K, Singh S, Singh S, Mishra S, Chauhan D K & Dubey N K, Micronutrients and their diverse role in agricultural crops: advances and future prospective, *Acta Physiol Plant*, 37 (2015) 139.
- Ayub M A, Naeem A, ur-Rehman M Z, Farooqi Z U R, Umar W, Fatima H, Nadeem M & Shabaan M, Role of nanotechnology in enhancing crop production and produce quality, *Sustain Nanotechnol Environ Remediat*, (2022) 703.
- Swify S, Mažeika R, Baltrusaitis J, Drapanauskaitė D & Barčauskaitė K, Modified urea fertilizers and their effects on improving nitrogen use efficiency (NUE), *Sustainability*, 16 (2023) 188.
- Savci S, Investigation of effect of chemical fertilizers on environment, *APCBEE Procedia*, 1 (2012) 287.
- Usman M, Farooq M, Wakeel A, Nawaz A, Cheema S A, ur-Rehman H, Ashraf I & Sanaullah M, Nanotechnology in agriculture: Current status, challenges and future opportunities, *Sci Total Environ*, 721 (2020) 137778.
- Perfileva A I & Krutovsky K V, Manganese nanoparticles: Synthesis, mechanisms of influence on plant resistance to stress, and prospects for application in agricultural chemistry, *J Agric Food Chem*, 72 (2024) 7564.
- Salama D M, Abd-El-Aziz M E, Osman S A, Abd-Elwahed M S A & Shaaban E A, Foliar spraying of MnO₂-NPs and its effect on vegetative growth, production, genomic stability, and chemical quality of the common dry bean, *Arab J Basic Appl Sci*, 29 (2022) 26.
- Sunil C, Kadam P V, Kanavi G B J, Onte S, Salimath S B, Jeevan H R, Mallikarjuna H B, Chandra M S & Seema N U, Comparative assessment of nano nitrogen and nano zinc nutrition on growth, yield and profitability of chilli (*Capsicum annum. L.*), *J Plant Nutr* (2024): 1-15.
- Salama D M, Abd-El-Aziz M E, Shaaban E A, Osman S A & Abd-El-Wahed M S, The impact of nanofertilizer on agro-morphological criteria, yield, and genomic stability of common bean (*Phaseolus vulgaris L.*), *Sci Rep*, 12 (2022) 18552.
- Ayenew B M, Satheesh N, Zegeye Z B & Kassie D A, A review on the production of nanofertilizers and its application in agriculture, *Heliyon*, 11 (2025) e41243.
- Haydar M S, Ghosh D & Roy S, Slow and controlled release nanofertilizers as an efficient tool for sustainable agriculture: Recent understanding and concerns, *Plant Nano Biology*, 7 (2024) 100058.
- Lindsay W L, *Chemical Equilibria in Soils*; Wiley-Interscience: New York, (1979).
- Fageria N K, Baligar V C & Clark R B, Micronutrients in crop production, *Adv Agron*, 77 (2002) 185.