JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/38685
RESEARCH ARTICLE

Impact of Engineered Metallic Nano-oxides on the Growth and Development of Medicinal Crop *Carthamus tinctorious* L

Anupriya Rana[1], Asha Sharma[2], Neha Kapoor[1] and Gaurav Sharma[1*]

[1]School of Applied Sciences, Suresh Gyan Vihar University, Jaipur - 302020, Rajasthan, India; gaurav.sharma@mygyanvihar.com

[2]Department of Zoology, Swargiya PNKS Government PG College, Dausa – 303303, Rajasthan, India

Abstract

Background: Nanotechnology has proved itself as a constructive as well as destructive step in the fields of medicine, agriculture, biosciences, pharmacology and engineering in the past three decades. As technology advances, so do diseases and other diverse inflammatory ailments in the modern world. Our daily health comes from the agricultural fields and surroundings. The increased metal toxicity and soil infertility are major causes of concern. **Aim:** This study aimed to analyse the growth requirements and impact of engineered metallic nano-oxide on the growth of a medicinal oil crop safflower (NARI -96) in a different but favourable geographical area (Rajasthan, India), unusual from its native geographical conditions. The major motive behind this study is to analyse the impact of metallic stress on yield and increase the crop production rate in Rajasthan for medicinal and economic benefit. **Methods:** The effect of discrete nano-oxides like silver, zinc, titanium and copper oxide with concentrations ranging from 00ppm to 80ppm have been evaluated on *Carthamus tinctorius* L. The study was conducted in a controlled environment in a Plant Tissue Culture (PTC) lab as well as in greenhouse conditions. **Results:** The foremost results in the PTC method have been found with the treatment of copper and zinc oxide. The silver oxide showed a toxic effect and retard the growth. The plant growth under normal environmental conditions showed different results due to the alkaline nature of the soil. The efficiency of titanium oxide and copper oxide has been showing better morphophysiological results in comparison to the control and other nano-oxides. **Conclusion:** The study reveals that metallic nano-oxides greatly influence the growth and development of safflower under controlled as well as in greenhouse conditions but the impact of copper oxide remains constant in both conditions.

Keywords: *Carthamus tinctorious*, Green House, Morphophysiological, Metallic Nano-oxides, Plant Tissue Culture

1. Introduction

Nanotechnology and metallic nano-oxides represent a significant advancement in the field of science and technology. This progress enables the development of distinct methodologies and tools for precise manipulation at the molecular and atomic scale. They also have the potential to reform the science of medicine and agriculture. Nanotechnology, its utilisation and its probable toxic effects on the ecology have created concerns about their usage[1]. Although apart from their adverse effects, Nano-Particles (NPs) are considered to be the materials of the new golden era in science and technology. The agricultural utility of advantageous nano-particles is presently an interesting and demanding field of research[2]. The present research studies the influence of heavy metals on the species of safflower. Heavy metals can be defined as types of metals that have a higher density of more than 5g/cm. Some of them like Ag, Zn, Fe, Ti, Mo, Ni and Cu are responsible for having a positive and negative impact. They are also required for the growth and development of plants. Metal nano-oxides or normal-size metals both dissolve easily and have the efficiency to transform. So they can easily be absorbed by the plants. The enhanced dissolution properties of ZnO and CuO

*Author for correspondence

Article Received on: 22.02.2024 Revised on: 11.06.2024 Accepted on: 25.06.2024

metallic nano-oxides have been observed, particularly impacting the germination of plants. During the sprouting of *Meicago sativas*, membrane lipids such as sulfolipids, phospholipids, and galactolipids have been noted to improve their functionality and increase the number of membrane folds. The linoleic fatty acid enriches their PC (Phosphatidylcholine) molecular species[6].

1.1 *Carthamus tinctorious*

The target species for research *C. tinctorious* L. commonly known as safflower is among the major Medicinal Oil Crops (MOC). The production of this crop is limited to Maharashtra, Telangana and Karnataka This study was designed in Rajasthan state to evaluate all possibilities of safflower growth and development. This will help to increase the production rate of such an important MOC, as the demand for oil crops is constantly increasing because of their abundant role in medicine, pharmacology, the food industry, bio-fuel production and also as animal feed. The initial purpose of cultivation of safflower was just limited to colouring and decorating in textile industries. Modern researchers have now discovered their exponential utility as an analgesic, antihypertensive and antithrombotic drug[7]. This species has attained importance in recent years as some discoveries reflect that safflower seed oil can be useful in the production and advancement of biodiesel[8].

Today, these oil crops occupy 11% of the total organic agricultural land which is almost 1.5 million hectares in area[9]. Safflower plants are quite drought-resistant and can tolerate a low groundwater level. This fact makes them different from other oil crops like soybeans, sunflower and rapeseed[10]. This unique character makes them ideal to grow in arid conditions like Rajasthan, India. The state has a low agricultural production rate due to harsh conditions, so increasing the production of beneficial crops can accelerate their economic conditions. To analyse all possible aspects of better growth, a detailed and comprehensive study was required.

1.2 Impact of Nano-oxides on Plants

Nutrient management is an important step or universal essentiality for upholding crop production and yield[11]. The changes in the composition of specific crop nutrition cause alterations in their physiological, chemical, morphological and anatomical structures. The rapid graph of metallic pollutants in the soil creates problems in the developmental studies of plants. Nano-materials have been considered noxious and cancer-causing and also cause different types of irritations as they can penetrate the cell membrane[12]. The present study includes the impact of engineered Metaloxides Nanoparticles (MONPs) like zinc, titanium, copper and silver on the plant growth of safflower under *in vitro* , PTC and normal environmental conditions. Every metal responds specifically to the species and also varies in results as positive, negative and neutral. The ZnO has the potential to be arranged as tetragonal complexes with N, O and majorly S ligands. This situation impacts the metabolic pathway. Zinc can also function as a catalyst in building and stimulating roles in various enzyme reactions inside the plants[13]. The ZnO NPs can also negatively affect the growth and biomass in *Arabidopsis*[14]. A smaller concentration of silver can show a significant growth impact on several plant species. It has been observed that the activity of certain antioxidant enzymes was stimulated in *Brassica juncea* seedlings which have been treated with silver nano particles[15]. The Safflower plant can reproduce in the same environment that favours crops like wheat and barley, but this crop has untapped potential with extensive compatibility16. The metal tolerance of safflower is greater compared to other fellow species. Safflower can tolerate the high concentration of zinc and copper without much alteration in its normal physiological functions[17]. Some research also shows that foliar spray of nano (Titanium Oxide) TiO impacts the traits and carotenoids, chlorophyll, soluble proteins and crop yield of safflower and the application of TiO enhanced physiological parameters of safflower[18].

2. Materials and Methods

2.1 *In vitro* Plant Growth

2.1.1 Plant Materials

The study was conducted at the Plant Tissue Culture lab at Suresh Gyan Vihar University Jaipur, India. The authorised Safflower (*NARI-96*) hybrids developed in 2018 with 2020 kg/h yield and 33% oil content[19] containing seeds have been collected from Krishi Vigyan Kendra (Government Agricultural sector) India. The seeds were sterilised by dipping in Iodine

0.1% $HgCl_2$ for almost 3 to 5 minutes and washed 10-15 times with distilled water.

2.1.2 Metallic Nano-oxides and Dose Preparations

The four engineered metallic nano-oxides, silver, zinc, titanium and copper were bought from a reputed industry Nano-Research Lab, Jharkhand. Their analysis (SEM and XRD) was studied carefully before studying their impacts on plants. The size of nano-oxides was between 30-50nm. The dose of nano-oxides was prepared with the combination of deionised water with powdery metallic nano-oxides. There was a total of 16 doses (4 metallic nano-oxides × 4 concentrations) of nano-oxides prepared. The nano-oxides cannot dissolve properly in water, so they always remain in the colloidal form. Every single time, when a dose needs to be given to a plant, a fresh colloidal solution of nano-oxides needs to be prepared. The weight of nano-oxides was taken very accurately with the digital weighing balance, as very small quantities of nano-oxide need to be added to make a solution of 20ppm, 40ppm, 60ppm, and 80ppm.

2.1.3 Media Preparation Along with Metallic Nano-oxides

Standard Murashige and Skoog Medium (MS) media have been made with different concentrations of metallic nano-oxides. Concentrations of every metallic nano oxide at specific measurements 20,40, 60 and 80 ppm) were added to the specific flask. The metallic particles have been added after sterilisation of media by auto-clave because of the uncertainty of reacting at high temperature and pressure. Those were added just before the inoculation process with vitamins and plant hormones like Kinetin (0.5 - 2.0mg dm^{-3}), NAA (0.5 - 1.0mg dm^{-3}), IAA (1.0mg dm^{-3}) and 2,4-D (0.44–85.62 μM)[20] necessary for the safflower plant growth and development.

2.1.4 Inoculation and Culturing

The seeds were cleansed with sterilising agents. The MS media were prepared with specific concentrations of metallic nano-oxides. The next step in the process was to inoculate the seeds carefully inside the media containing flasks without contaminating them. The laminar flow was prepared for the inoculation by sterilising the surfaces and turning on the flame lamp. The inoculation was followed by placing a pointed side of the seed inside the media and a broadside half above it. The process was then continued by transferring the flask inside the culture room with specific favourable conditions for safflower growth. The conditions were 23±2°C temperature and 63-70% humidity with a photoperiod of 16/8 hours.

2.1.5 Analysis of Germination, Plant Growth and Hardening

The seeds were placed in a flask with specific concentrations of metallic nano-oxides. The cultures were monitored twice a day. The results were observed in the form of the effect of metallic nano-oxides on the earliest to late seed germination, shoot and root length and capacity of the plant to grow in normal or outward conditions after hardening. Hardening is the process of transfer of PTC plants from a controlled environment to outer or under normal environmental conditions. The plant growth was monitored till the 3rd month from the day of hardening.

2.2 Green House Study

2.2.1 Field Work

A greenhouse was prepared in December as it was the ideal time for safflower germination in Rajasthan, India. Four separate rows comprising 16 treatments (4 nano-oxides × 4 concentrations) and 2 controls were prepared for studying the impacts of metallic nano-oxides on the morphophysiological aspects of safflower. Those were replicated in triplet for unbiased results. The distance between the plants was 0.7 meters which was enough for root growth and development.

2.2.2 Soil Health Management

The soil of Jaipur, Rajasthan, India is alkaline in nature. The measured pH of the soil was 8.8 on the scale. The Nitrogen, Phosphorus and Potassium (NPK) proportion was also medium and not enough for healthy safflower growth[21]. To make it more fertilised and slightly acidic, an optimum amount of sphagnum peat and vermicompost was been added to the soil. The Soil composition was checked for optimum fertility. The soil conditions were monitored till harvesting.

2.2.3 Nano-dose Preparation and Implication

The doses of metallic nano-oxides were prepared in the same manner as they were prepared for the *in vitro* studies. The prepared fresh colloidal solution of metallic nano-oxides was given to the plants as a foliar spray on their leaves and spraying solution on their roots. The foliar and root spray will help plants to directly intake the metallic nano-oxides through stomata and root hairs. The doses were sprayed twice a month from germination to flowering. 10ml of dose solution was sprayed on each plant's root and branches.

2.2.4 Monitoring the Growth and Developmental of Plant Vegetative and Reproductive Phases

Growth was first monitored at the time of germination. The germination time of every plant was noted with the length of the germinated plant. On every sixth day, plants' condition was monitored and analysed in terms of plant height, colour, leaf count, diameter, healthiness, pattern of leaf arrangement, bud formation, flowering stage, flower size, seed formation and also any type of disease symptoms. Every stage and activity of the plant was carefully examined and noted on time. The quality of agronomy was maintained with quality irrigation and organic fertilizers.

2.2.5 Seed Collection and Storage

The process of seed collection was initiated after the ripening of the flower. The stage of seed collection is reached when the flower becomes yellow or brown and all the petals fall. The morphological study of seeds was done carefully to analyse the minimum to maximum impact of metallic nano-oxide. To get an effective seed morphological analysis four to five parameters have been evaluated, like weight, colour, texture, size and thickness of the seeds. The flowers were collected and stored in small polythene pouches to keep them separate and safe from moisture.

3. Results

3.1 *In vitro* Analysis of Metallic Nano-oxides on the Growth of Safflower

3.1.1 Metallic Nano-oxides Influence on Germinations

The influence of MONPs on germination has been seen in many previous studies as the germination activities have been monitored from the 4^{th} day when the earliest and best seed germinates from a 20ppm concentration of TiO-treated plant. (Figures 1 and 3) The TiO showed a positive influence on the germination up to 40ppm. The zinc and CuO showed moderate growth and almost all concentration treatments germinated by the 6^{th} day (Figure1). The AgO germination efficiency was not quite up to mark. The 20ppm and 40ppm grew by the 7^{th} day but most of the seeds from 60 and 80ppm did not germinate properly due to increased toxicity of AgO. The germination sustainability of ZnOs treated seeds was the highest (Figure 1).

3.1.2 Influence of Metallic Nano-oxides on Morphology

The morphological parameters have been measured in terms of root, shoot health and their length. Exponential growth has been seen at every concentration of copper-oxide treatment from 20ppm to 80ppm (Figures 1 and 4, Tables 1 and 2). Minimum growth has been seen in the concentrations of silver-oxide. The effect of zinc was moderate and showed the best results at a concentration of 20 and 60 ppm. The initial concentrations of titanium-oxide also performed well on morphological features of safflower

Table 1. Effect of specific metallic nano-oxides concentrations on shoot length (cm) of safflower

Nano-oxides	Control(cm)	20ppm(cm)	40ppm(cm)	60ppm(cm)	80ppm(cm)
ZnO	1.43± 0.08	1.83±0.03	1.53±0.23	1.70±0.20	2.26±0.08
TiO	1.43±0.08	2.80±0.05	1.73±0.27	1.46±0.21	1.06±0.06
AgO	1.43±0.08	1.26±0.03	0.86±0.03	1.20±0.05	0.96±0.08
CuO	1.43±0.08	2.33±0.08	2.03±0.34	1.33±0.14	1.70±0.45

*±Average value with standard error, ANOVA: two factors with replication test with significant p-value (0.03- 0.05%), ZnO-Zinc oxide, TiO-Titanium oxide, AgO–Silver Oxide, CuO-Copper oxide. Measurements have been taken in centimetres.

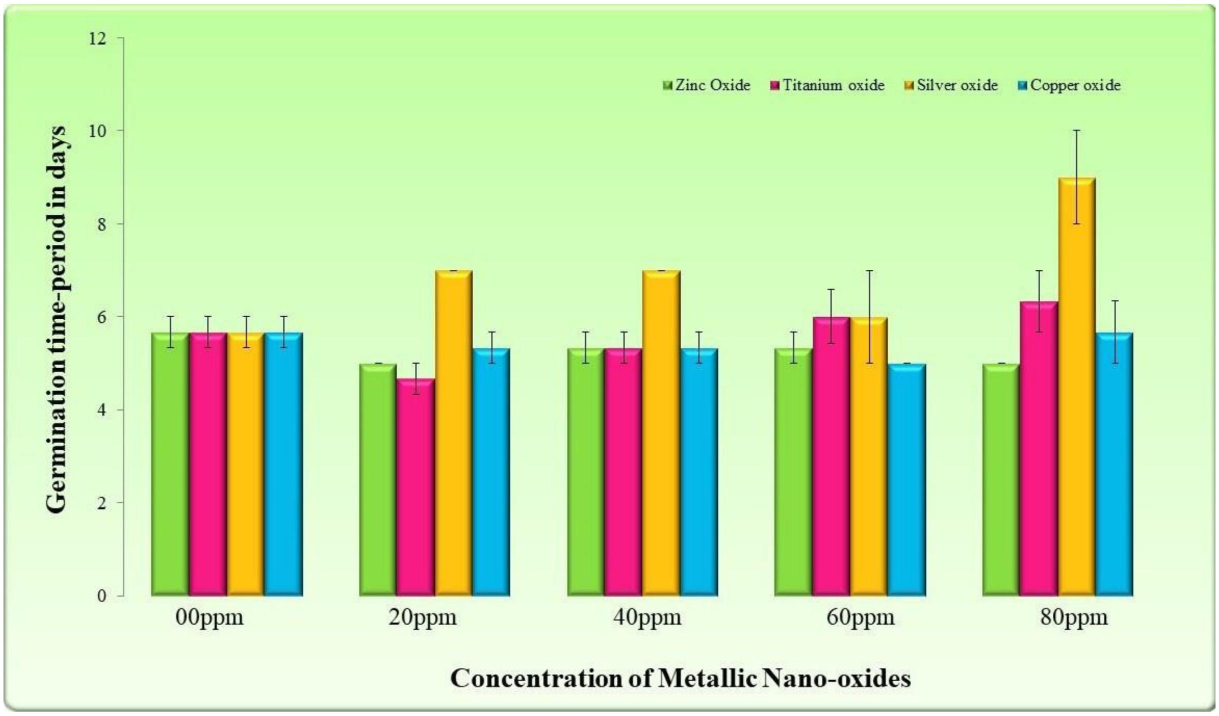

Figure 1. Germination effect on safflower seeds under the influence of specific metallic nano-oxides concentration.

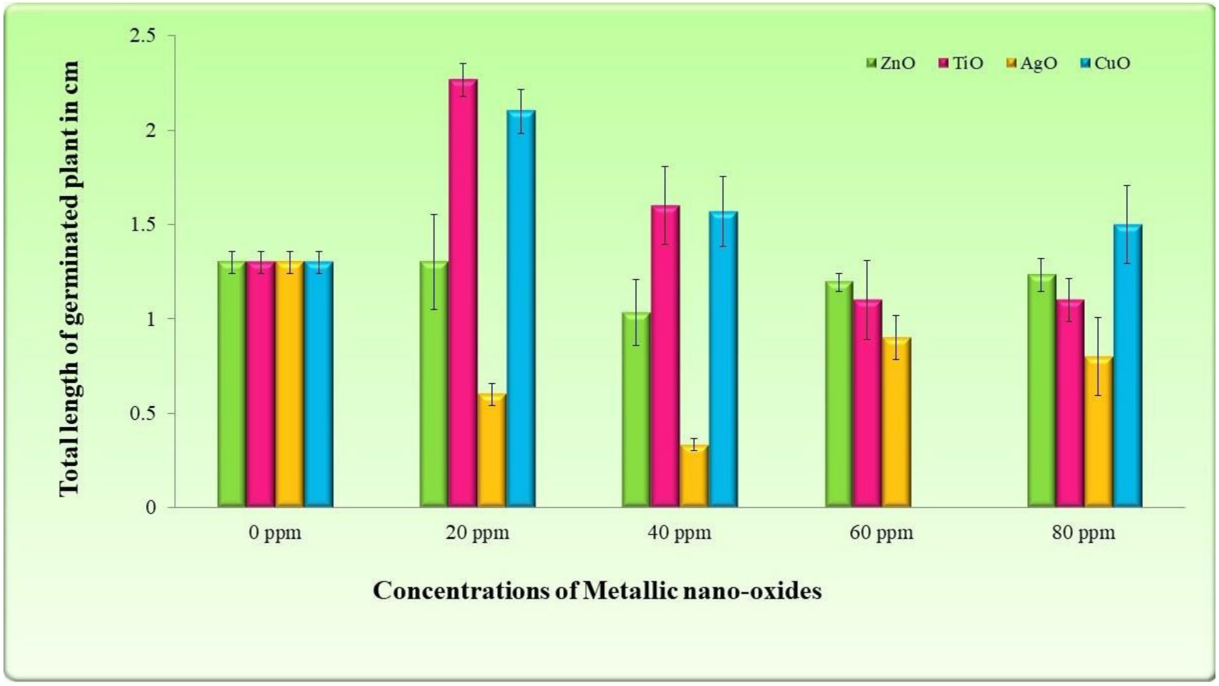

Figure 2. Effect of metallic nano-oxides on the total length of safflower seedlings on the 14th day after inoculation.

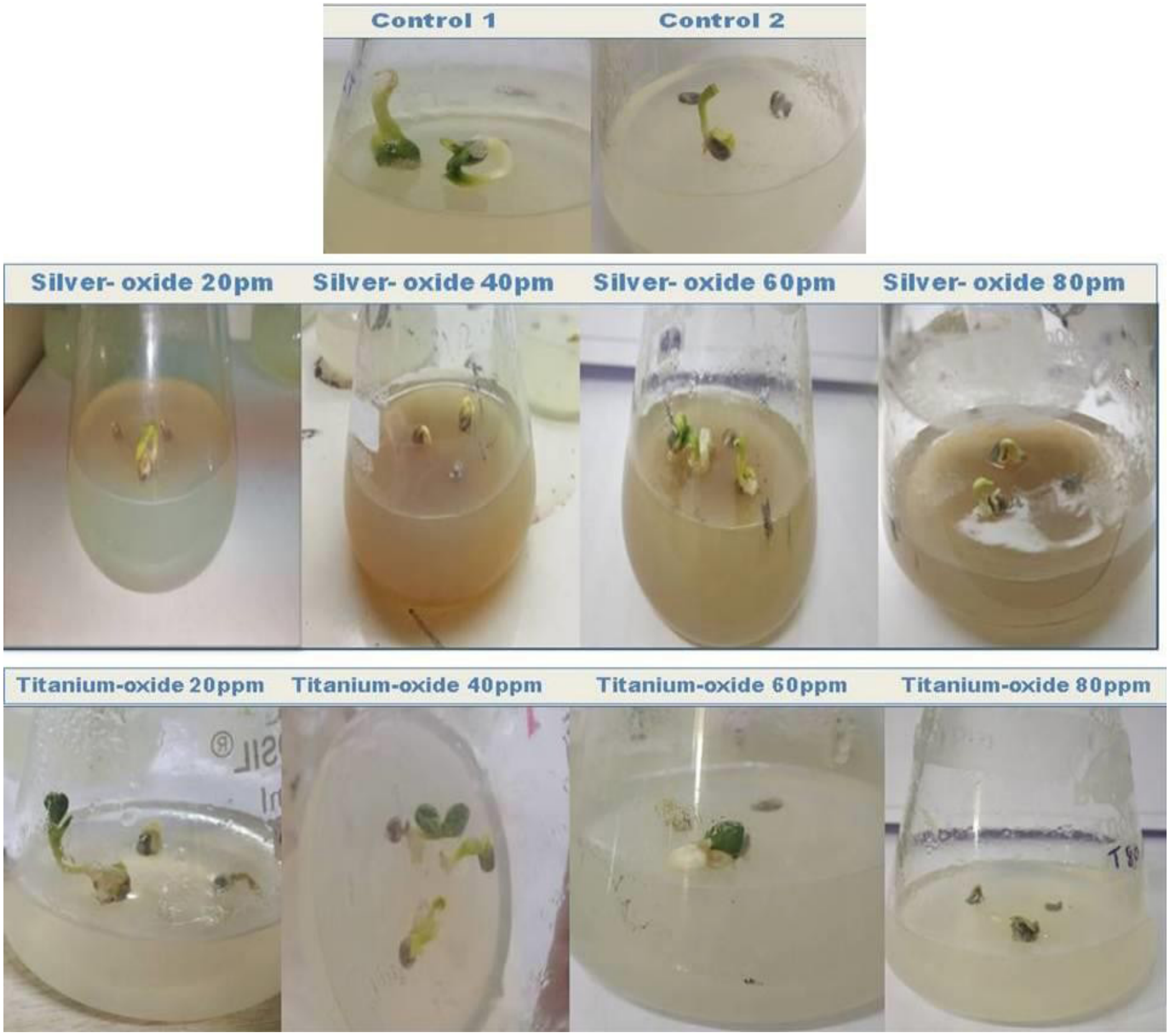

Figure 3. Impact of specific concentration of silver and titanium nano-oxide on the seed germination of safflower under *in vitro* conditions.

Table 2. Effect of specific metallic nano-oxides concentrations on the root length (cm) of safflower

Nano-oxides	Control(cm)	20ppm(cm)	40ppm(cm)	60ppm(cm)	80ppm(cm)
ZnO	1.00±0.05	1.46±0.08	1.00±0.1	1.26±0.03	1.63±0.03
TiO	1.00±0.05	1.73±0.06	1.43±0.17	1.03±0.12	0.80±0.05
AgO	1.00±0.05	0.96±0.12	0.40±0.05	0.76±0.18	0.86±0.03
CuO	1.00±0.05	1.33±0.13	1.56±0.17	1.03±0.12	1.10±0.15

but 60 and 80ppm concentrations were malignant (Figures 2 and 3, Tables 1 and 2). The fresh and long life of plants was best seen at zinc and copper 20ppm concentration, This concludes that mostly initial or very little dose of nano-oxide has been favourable for the growth of safflower in comparison to high concentrations.

3.1.3 Hardening

Hardening or placing *in vitro* plants to outer harsh conditions is a challenge for their sustainable growth and development. The hardening process was done after 14 days of *in vitro* growth. The plants were shifted from the PTC lab to pots under normal environmental conditions (Table 3).

Figure 4. Impact of specific concentration of zinc and copper nano-oxide on the seed germination of safflower under *in vitro* conditions.

Table 3. Effect of specific metallic nano-oxides on the lifespan (days) of safflower after hardening

Nano-oxides	Control	20ppm	20ppm	40ppm	60ppm	80ppm
ZnO	49.00±1.73	72.33±6.69	72.33±6.69	37.66±3.48	40.00±4.04	63.00±7.50
TiO	49.00±1.73	50.66±1.20	50.66±1.20	45.33±0.88	40.00±3.78	35.00±1.52
AgO	49.00±1.73	12.66±1.20	12.66±1.20	17.33±2.02	23.00±2.64	28.00±2.51
CuO	49.00±1.73	72.66±4.63	72.66±4.63	47.33±11.28	45.66±0.33	46.00±1.52

±Average value with standard error, ANOVA: two factors with replication test with significant p-value range (0.03- 0.05%).

3.2 Green House Analysis of Metallic–nano-oxides on the Growth of Safflower

3.2.1 The Impact and Assessment of Metallic Nano-oxide on the Growth of Carthamus tinctorious (Safflower)

Several parameters for growth and development have been considered in the form of germination studies such as the height of the plant, leaves count, leaf type, bud timing, number of flowers per plant, apical and lateral flowers, seeds per flower, flower diameter, seed morphology and seed yield.

3.2.2 Metallicnano-oxide Impact on the Germination of Safflower

The very first or the earliest seedling appeared on Titanium 20ppm on the sixth day after seeds owing followed by 80 and 60ppm of TiO, CuO 20 and 40ppm, AgO 80ppm and lastly in ZnO treated plants. A delayed germination has been noticed in Zinc 60, copper 80 and silver 60ppm. They all take a little longer than expected in comparison to control and other oxide concentrations. All the TiO-treated plants were grown on or before time and remained healthy and heightened at their juvenile stage (Table 4).

3.2.3 Metallic Nano-oxide Impact on the Height of Safflower

The height is an important parameter in the growth and development of any plant. The correlation of height with other factors has been unknown in many plants. During the assessment of metallic nano-oxide, very little correlation has been observed among the various concentrations of nano-oxides. The metallic nano-oxide affects the height of the safflower. The tallest plant has been seen at the concentration of TiO 40ppm. A better impact of ZnO nano-particles in comparison to control has been seen on the lower concentrations like 20ppm and 40ppm but as the concentration of ZnO increased, the height of plants decreased. The effect of AgO has been raised with the concentration as 20ppm showed very little growth in comparison to control but the concentration of 80ppm showed better growth in the height of the plant. The CuO did not show much growth in the height of the plants (Table 5).

3.2.4 Metallic Nano-oxide Impact on the Leaf Count of Safflower

The impact of a metallic oxide is also evaluated on the leaf count of safflower. The foliage determines the photosynthetic capacity of any plant because that is considered an important parameter to evaluate the growth and development of the plant. Overall, if we see the effect of metallic nano-oxide; there have been no significant differences in leaf count on lower np concentrations. Specifically, the effect of the lower concentrations of AgO neither gave any positive nor negative results. The 80ppm concentration showed a little better leaf count in comparison to other concentrations and also control. The ZnO showed better results in lower concentrations but higher concentrations did not show any better results. Their leaf count was also very low. The plants that were treated with TiO showed better results in comparison to the control, the maximum foliage was reported on the 40ppm concentration of TiO and the minimum foliage was seen on the 60ppm concentration of ZnO. The effect of CuO showed better results at the concentrations of 20 and 40 ppm. The rest of the concentrations remained the same as the control (Table 6).

Table 4. Effect of metallic nano-oxide on the germination of safflower seeds (out of triplets % of germination) under greenhouse conditions

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	0.36±0.013	1.20±0.01	1.46±0.023	1.50±0.04	1.67±0.043
ZnO	0.36±0.013	1.03±0.023	0.96±0.023	0.40±0.01	0.30±0.07
AgO	0.36±0.013	0.30±0.01	0.23±0.063	0.73±0.14	1.23±0.023
CuO	0.36±0.013	1.13±0.023	1.30±0.023	0.83±0.003	0.73±0.023

Table 5. Effect of metallic nano-oxides on the height (cm) of safflower under greenhouse condition

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	59.66±9.33	59.33±4.33	118.66±4.33	97.66±2.33	106.33±25.33
ZnO	59.66±9.33	95.00±4.00	96.66±6.33	59.66±2.33	14.66±2.11
AgO	59.66±9.33	27.66±2.33	42.33±10.33	64.66±12.33	93.00±4.00
CuO	59.66±9.33	70.33±2.33	85.33±12.33	48.33±2.33	49.33±2.33

3.2.5 Metallic Nano-oxide Impact on the Timing of Bud Formation in Safflower

Bud formation is a very primary step towards the reproductive phase of the plant. The beginning of a reproductive phase in safflower starts with the aggregation of fertile leaves which come together at the very apical portion of apical branches. There are two types of branches present in the safflower. One is the apical branches and the other is the lateral branches. The apical branches are designed to produce buds but lateral buds also develop in many plants. The bud formation takes place in almost the third month of plant growth and so the control attains the bud formation on the 89th day of plant growth. Some of the metallic oxides treated plants have an impact on bud formation by causing early bud formation. Some of the plants, however, showed a slight delayed bud formation. The impact of metallic nano-oxides and the very earliest bud formation has been seen in TiO 60ppm concentration on the78th day. All the TiO-treated plants showed early bud formation in comparison to the control. The effect of ZnO 60 and 80ppm showed a little late bud formation but AgO20 and 40ppm were equivalent to control (Table 7).

3.2.6 Impact of Metallic Nano-oxide on the Total Numbers of Flowers and Apical Flowers

The flower is the most important part of the plant. When bud formation occurs in plants, only after that are they assumed to complete their life cycle. The number of flowers also determines and gives an idea of the seed yield of a particular plant. The control had flowers between two to three. A major number of apical and terminal flowers has been observed in the case of TiO 60PPM and the lowest number of the flowers has been reported on the zinc oxide 60PPM concentration. The AgO and CuO did not show a very significant difference in the flower number except for the AgO 80ppm and CuO 40ppm (Table 8). Major seed yield was monitored by having a maximum number of apical flowers. There have been positive and negative effects of the treatment of metallic oxide on apical flower count. The plants treated with ZnO resulted in single apical flowers except the 20ppm. AgO treatment also showed one single flower, except the 80ppm concentration which showed three apical flowers. The results of copper and TiO were similar to the control, except the 40ppm of CuO and the 60ppm of TiO which showed better results in comparison to the control (Table 9).

Table 6. Effect of metallic nano-oxides on the leaves count of safflower under greenhouse condition

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	41.33±14.33	43.66±0.33	56.33±1.33	45.66±2.33	52.66±4.33
ZnO	41.33±14.33	48.00±1.00	49.00±1.00	20.00±1.00	29.33±4.33
AgO	41.33±14.33	38.66±2.33	39.66±2.33	42.00±1.00	47.33±2.33
CuO	41.33±14.33	48.00±1.00	51.00±1.00	39.33±0.33	41.00±1.00

Table 7. Effect of metallic nano-oxides on the bud timing (days) of safflower under greenhouse condition

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	89.33±2.33	81.00±1.00	81.33±0.33	78.00±1.00	79.66±4.33
ZnO	89.33±2.33	87.66±0.33	84.66±0.33	91.00±1.00	93.00±7.00
AgO	89.33±2.33	92.00±1.00	89.66±0.33	89.33±0.33	85.00±4.00
CuO	89.33±2.33	85.33±0.33	84.66±1.33	89.00±1.00	91.00±1.00

*Bud Time in Days

Table 8. Effect of metallic nano-oxides on the number of flowers of safflower during greenhouse condition

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	2.66±0.33	4.00±1.00	7.00±1.00	8.66±1.33	6.66±0.33
ZnO	2.66±0.33	2.66±0.33	4.33±0.33	1.66±0.33	1.66±0.33
AgO	2.66±0.33	1.66±0.33	3.33±0.33	2.66±0.33	5.33±0.33
CuO	2.66±0.33	4.33±0.33	5.33±2.33	3.66±0.33	3.33±0.33

Table 9. Effect of metallic nano-oxides on the number of apical flowers in safflower under Greenhouse condition

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	2.33±0.33	1.66±0.33	3.00±00	4.33±0.33	4.00±00
ZnO	2.33±0.33	1.66±0.33	1.33±0.33	1.00±00	1.00±00
AgO	2.33±0.33	1.33±0.23	1.66±0.33	1.66±0.33	3.33±0.33
CuO	2.33±0.33	2.33±0.33	3.66±0.54	3.33±0.33	2.33±0.33

3.2.7 Metallic Nano-oxide Impact on the Flower Diameter of Safflower

The diameter of the flower determines or can predict the expected seed yield. The control plant had a diameter of 4.5cm. Among all metallic nano-oxide treatments some specific concentrations gave better flower diameters otherwise rest showed equivalent or lower diameters in comparison to the control. The concentrations of 40, 60 and 80ppm of titanium and silver metallic nano-oxide treated plants produced better flower diameter in comparison to control. The correlation between the safflower flower diameters and seed yield was not related (Table 10).

3.2.8 Metallic Nano-oxide Impact on the Number of Seeds in a Single Flower

The impact of metallic nano-oxide showed great variance in the number of seeds in a single flower. The average number of seeds in a safflower flower is between 10 and 30 and the planted control got an average of 12 seeds in a flower. Maximum yield from a single flower has been seen on the plants treated with TiO 40 and 60ppm. The ZnO has shown a negative impact on the seed count except 40ppm. The copper-treated flowers also showed a better and higher yield of seeds in them (Figure 5).

3.2.9 Metallic Nano-oxide Impact on the Seed Yield and Seed Weight of Safflower

The earlier stated statement about expecting the seed yield from the diameter of the flower does not apply in the case of safflower. There has been a significant difference between the control and the plants treated with metallic nano-oxide. The girth of the flower was majorly filled with lower floret. The control had only 32 seeds in the entire plant in comparison to TiOtreated plants which showed better results. The best seed yield was obtained from the concentration of 40 to 80ppm of TiO. The result of ZnO treatment was not very effective on seed yield except the 40ppm concentration. The treatment with AgO nanoparticles showed better results on every concentration except the 20ppm of AgO. The treatment effect of CuO showed better results in comparison to the control (Figure 6). The seed yield is the total amount of seeds present in a particular plant. The results revealed that seed yield is not very much related to the weight of the seeds. Some seeds were very light in weight and almost hollow. The maximum yield has been seen at the concentration of TiO 60ppm and CuO 40ppm, Both of these concentrations had a huge difference in seed yield in comparison to control but did not maintain the same difference in the case of seed weight. The minimum seed weight was obtained

Table 10. Effect of metallic nano-oxide on the flower diameter (cm) of safflower flowers under greenhouse conditions.

Nano-oxide	Control	20ppm	40ppm	60ppm	80ppm
TiO	4.50±0.13	3.5±0.25	5.83±1.08	6.50±0.25	5.43±0.14
ZnO	4.50±0.13	4.33±2.30	6.16±0.58	2.00±0.25	4.00±0.25
AgO	4.50±0.13	3.16±0.58	5.40 ±0.03	5.03±0.66	4.90±0.93
CuO	4.50±0.13	4.81±0.00	4.90±0.13	4.73±0.25	4.96±0.02

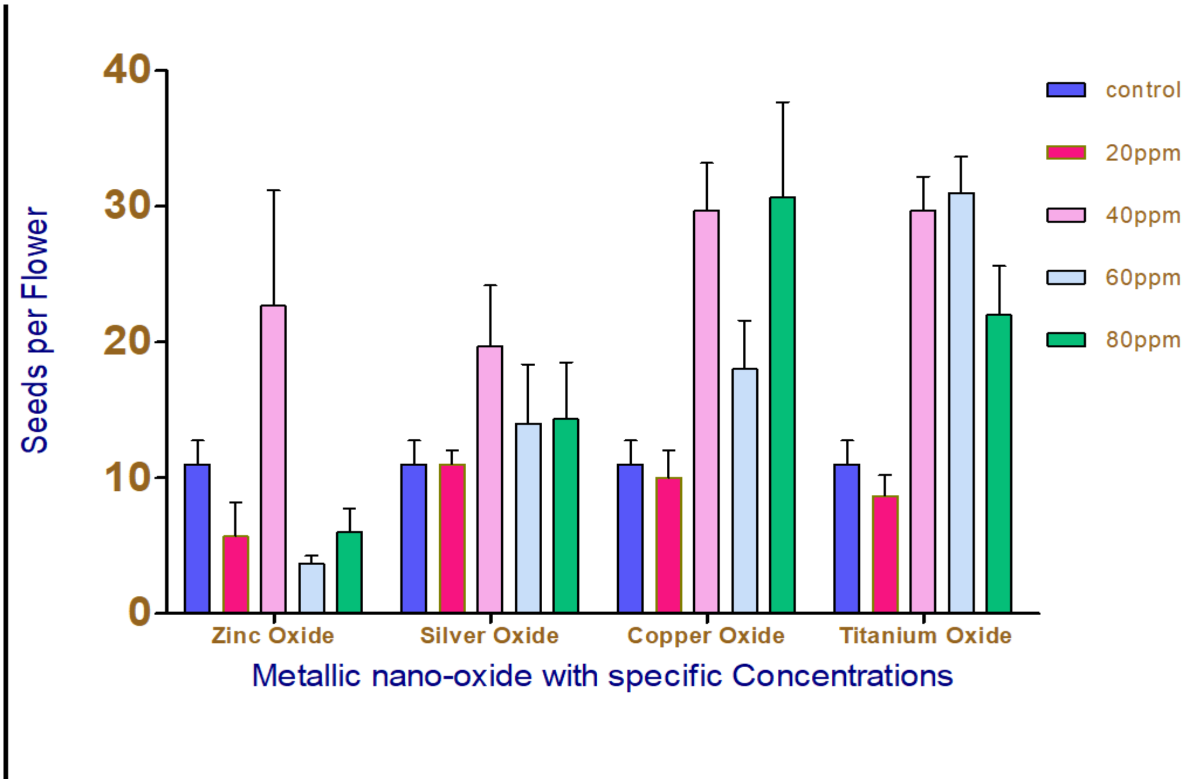

Figure 5. The effect of metallic nano-oxides on the seed yield of a single flower.

with the ZnO treatment at 20ppm, 60ppm and 80ppm concentrations (Figure 7).

4. Discussions

The impact analysis of metallic nano-oxides has been studied in many medicinal plants and crops. Several previous *in vitro* studies also prove the capability of TiO in better seed germination as the vigour index was increased in fennel seeds treated with TiO_2 NPs, as compared to the bulk TiO_2-treated plants and control[22,23]. The seeds from 60 and 80ppm treated MNOPs did not germinate properly due to the increased toxicity of AgO. The toxicity of silver at a dose of 0.2, 0.5 and 1mg L−1 for 7 days, has also been seen on root and shoot biomass as well as the root development and elongation in rice seedlings[24].

The morphology of plants is affected by the treatment of metallic NPs like ZnO, Fe_2O_3, Al_2O_3 and CuO NPs in various crop systems[25,26]. The basic reason behind it is due to the variation in nutrition uptake in plant systems in response to an increase or decrease in metallic stress[27]. The growth results of every plant after hardening were divergent. Some of them especially the ones treated with silver died very early as the silver NPs made their place in plasmodesmata and the cell

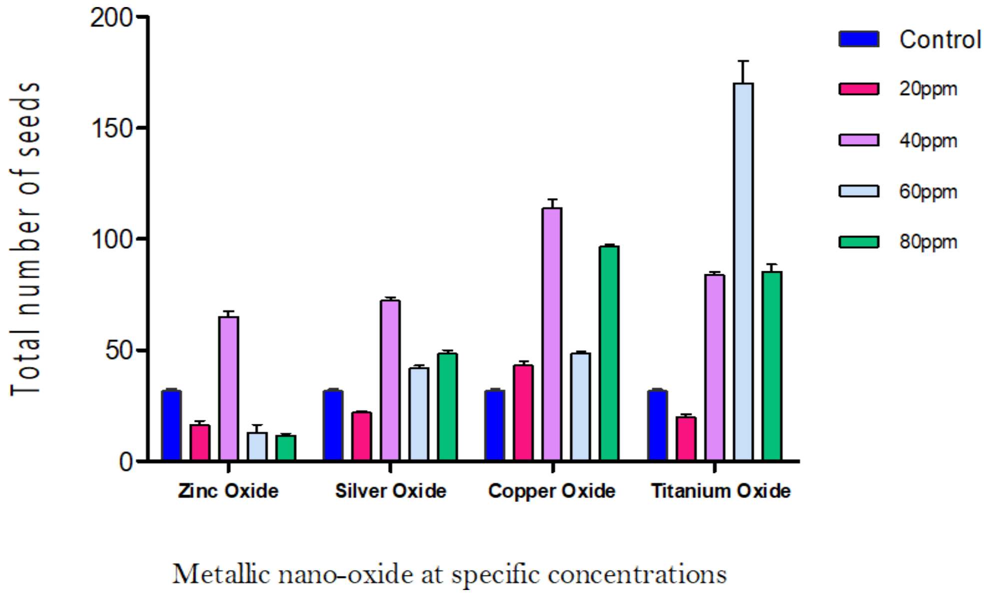

Figure 6. The effect of metallic nano-oxide on the total seed yield of safflower plants.

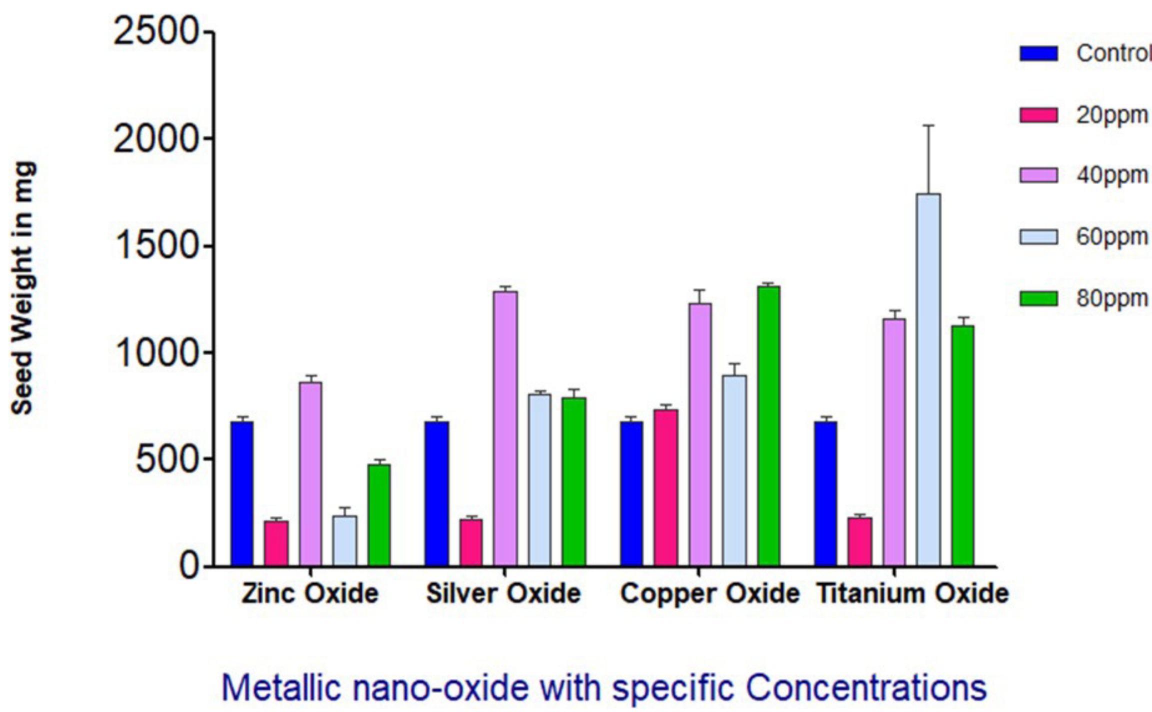

Figure 7. The effect of metallic nano-oxides on the total seed weight/ mass of safflower plants.

wall[28], which would result in wall seepage and clog the intercellular communication[29], sequentially contorting the activity of intercellular transport nutrients and transporter proteins thus resulting in a retarded growth of the plants[30]. Few plants that were treated with TiO stayed a bit longer and completed the vegetative phase but didn't make it to the reproductive phase. The plants which were treated with zinc and CuO spent their entire life cycle without having any major pitfalls.

The efficiency and benefits of TiO in normal environment seed germination and plant growth have already been stated in many previous studies as the nitrate reductase enhanced by the treatment with nano TiO in the process of soybean seed germination[31]. Treatment with TiO Nps also improves the growth as well as the yield of wheat plants even in water-deficit conditions[32]. The CuO facilitates the growth of safflower at most treatments in comparison to the control. The 5, 10 and 20mg L−1 doses of CuO NPs also increased the content of nutrients, like Cu, P and S in *Medicago sativa* shoots[33].

5. Statistics

The data has been statistically approved by applying the test two-way ANNOVA (Analysis of Variance) statistically significant between the means of three replications or more independent groups with two-factor factor replications. The test was applied through the software Graph Pad Prism and some tables were prepared in Excel software. The average value with standard deviations has been presented in the tables and graphs. The results were significant with a 5% probability value. The p-value < 0.05 was considered statistically significant.

6. Conclusion

The *in vitro* studies of safflower under the influence of specific concentrations of metallic nano-oxides reveal that controlled treatment of copper and ZnO can enhance the growth, morphological features and life span of safflower. The toxic effects of AgOs have been seen even at lower concentrations like 20 and 40ppm. The germination effect of TiO has been best at lower concentrations but it did not maintain the same level subsequently. The greenhouse studies showed the earliest germination at TiO 60 and 80ppm concentrations. The tallest plant has been measured at the titanium 40ppm concentration. Each concentration of TiO showed better foliage in comparison to the control. The early bud formation occurred in all silver and TiO-treated plants in comparison to the control. The maximum number of flowers was present on all the TiO-treated plants. The highest number of the apical flower has been seen on the TiO 60ppm. The seed yield of TiO, AgO and CuO were better in comparison to control. The ZnO treatment gave negative results in most of the parameters.

7. Acknowledgement

The authors are grateful to Krishi Vigyan Kendra (Government Agricultural Sector) India for providing Safflower seeds.

8. References

1. Chang YN, Zhang M, Xia L, Zhang J, Xing G. The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials. 2012; 5(12):2850-71. https://doi.org/10.3390/ma5122850 PMCid:PMC5449046.
2. Karunakaran G, Suriyaprabha R, Manivasakan P, Yuvakkumar R, Rajendran V, Prabu P, Kannan N. Effect of nano silica and silicon sources on plant growth promoting rhizobacteria, soil nutrients and maize seed germination. IET Nanobiotechnol. 2013; 7(3):70-7. https://doi.org/10.1049/iet-nbt.2012.0048 PMid:24028804.
3. Martínez-Fernández D, Barroso D, Komárek M. Root water transport of *Helianthus annuus* L. under iron oxide nanoparticle exposure. ESPR. 2016; 23:1732-41. https://doi.org/10.1007/s11356-015-5423-5 PMid:26396006.
4. Zafar H, Ali A, Zia M. CuO nanoparticles inhibited root growth from *Brassica nigra* seedlings but induced root from stem and leaf explants. Appl Biochem Biotechnol. 2017; 181:365-78. https://doi.org/10.1007/s12010-016-2217-2 PMid:27562818.
5. Rajput VD, Tstitsuashvili VS, Sushkova SN, Nevidomskaya DG. Effects of CuO nanoparticles on soil, plant and microbial community. International Scientific Conference XX Dokoutchaev Youth Readings, St. Petersburg, Russia.2017; .p. 12 https://elibrary.ru/item.asp?id=42849072
6. Li R, He J, Xie H, Wang W, Bose SK, Sun Y, Hu J, Yin H. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Int J Biol Macromol. 2019; 126:91-100 https://doi.org/10.1016/j.ijbiomac.2018.12.118 PMid:30557637.
7. Nagaraj B, Malakar B, Divya TK, Krishnamurthy N, Liny P, Dinesh R, Iconaru S, Ciobanu C. Synthesis of plant-mediated gold nanoparticles using flower extracts of *Carthamus tinctorius* L.(safflower) and evaluation of their biological activities. Dig. J. Nanomater. Biostruct. 2012; 7:1289-96. https://chalcogen.ro/1289_Nagaraj.pdf
8. Willer La Bella S, Tuttolomondo T, Lazzeri L, Matteo R, Leto C, Licata M. An agronomic evaluation of new safflower (*Carthamus tinctorius* L.) germplasm for seed and oil yields under Mediterranean climate conditions. Agronomy. 2019; 9(8):468. https://doi.org/10.3390/agronomy9080468
9. Willer H, Travnicek J, Schlatter B. Current status of organic oilseeds worldwide–statistical update. Oilseeds and Fats Crops and Lipids. 2020; 27:62. https://doi.org/10.1051/ocl/2020048

10. Razumnova LA, Kamenev RA, Turchin VV. Effect of mineral fertilizers and bacterial preparations on the productivity and oil content of safflower in the North-Eastern area of the Rostov region. Bull Voronezh State Agrarian Univ. 2018; 1(56):43-9. https://doi.org/10.1051/e3sconf/202451001010
11. Meharg A. Marschner's mineral nutrition of higher plants. Edited by P. Marschner. Amsterdam, Netherlands: Elsevier/ Academic Press. 2011; p. 684. ISBN 978-0-12-384905-2. Exp Agric. 2012; 48(2):305 https://doi.org/10.1017/S001447971100130X
12. Kumar H, Venkatesh N, Bhowmik H, Kuila A. Metallic nanoparticle: a review. Biomed. J. Sci. Tech. Res. 2018; 4(2):3765-75. https://doi.org/10.26717/BJSTR.2018.04.0001011
13. Prasad TN, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T. Effect of nanoscale ZnO particles on the germination, growth and yield of peanut. J Plant Nutr. 2012; 35(6):905-27. https://doi.org/10.1080/01904167.2012.663443
14. Wang X, Yang X, Chen S, Li Q, Wang W, Hou C, Gao X, Wang L, Wang S. ZnO nanoparticles affect biomass accumulation and photosynthesis in Arabidopsis. Front Plant Sci. 2016; 6:1243. https://doi.org/10.3389/fpls.2015.01243
15. Sharma P, Bhatt D, Zaidi MG, Saradhi PP, Khanna PK, Arora S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotechnol. 2012; 167:2225-33. https://doi.org/10.1007/s12010-012-9759-8 PMid:22692847.
16. Gilbert J, Knights SE, Potter TD. International safflower production-an overview. In International Safflower Conference. Australian Oilseeds Federation. Wagga Wagga, Australia 2008; https://s3.wp.wsu.edu/uploads/sites/2171/2017/11/Keynote-Gilbert-keynote-paper.pdf.
17. Sayyad G, Afyuni M, Mousavi SF, Abbaspour KC, Hajabbasi MA, Richards BK, Schulin R. Effects of cadmium, copper, lead and zinc contamination on metal accumulation by safflower and wheat. Soil Sediment Contam. 2009; 18(2):216-28. https://doi.org/10.1080/15320380802660248
18. Morteza E, Moaveni P, Morteza T, Saemi H, Joorabloo A. Effects of TiO2 (nano and bulk) foliar application on physiological traits and grain yield of safflower (*Carthamus tinctorius* L.). Biol Forum. 2015; 7(1):1725-31. Satya Prakashan. http://researchtrend.net/bf12/273%20ELHAM%20MORTEZA.pdf
19. Singh V. Safflower research at the Nimbkar Agricultural Research Institute (NARI). Phaltan-415523, Maharashtra, India. 2018. https://nariphaltan.org/safflowerhighlights.pdf.
20. Lijiao FA, Meili GU. Progress of safflower (*Carthamus tinctorius* L.) regeneration through tissue culture. Journal of Medical Colleges of PLA. 2013; 28(5):289-30. https://doi.org/10.1016/S1000-1948(13)60045-3
21. Krishi Vigyan Kendra, Jaipur 1, Soil analysis department, District Profile. (http://jaipur1.kvk2.in/district-profile.html).
22. Feizi H, Rezvani Moghaddam P, Shahtahmassebi N, Fotovat A. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. Biol Trace Elem Res. 2012; 146:101-6. https://doi.org/10.1007/s12011-011-9222-7 PMid:21979242.
23. Verma SK, Das AK, Patel MK, Shah A, Kumar V, Gantait S. Engineered nanomaterials for plant growth and development: a perspective analysis. Sci. Total Environ. 2018; 630:1413-35. https://doi.org/10.1016/j.scitotenv.2018.02.313 PMid:29554761.
24. Nair PM, Chung IM. Impact of CuO nanoparticles exposure on *Arabidopsis thaliana* growth, root system development, root lignification, and molecular level changes. Environ Sci Pollut Res Int. 2014; 21:12709-22. https://doi.org/10.1007/s11356-014-3210-3 https://doi.org/10.1007/s11356-014-3210-3 PMid:24965006
25. Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, Boyanov MI, Anderson AJ. CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. J Nanopart Res. 2012; 14(9):1-15. https://doi.org/10.1007/s11051-012-1125-9
26. Mahmoodzadeh H, Aghili R, Nabavi M. Physiological effects of TiO2 nanoparticles on wheat (*Triticum aestivum*). TJEAS. 2013; 3(14):1365-70. http://tjeas.com/wp-content/uploads/2013/08/1365-1370.pdf
27. Gopalakrishnan Nair PM, Kim SH, Chung IM. CuO nanoparticle toxicity in mung bean (*Vigna radiata* L.) seedlings: physiological and molecular level responses of *in vitro* grown plants. Acta Physiol Plant. 2014; 36(11):2947-58. https://doi.org/10.1007/s11738-014-1667-9
28. Geisler-Lee J, Wang Q, Yao Y, Zhang W, Geisler M, Li K, Huang Y, Chen Y, A, Ma X. Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. Nanotoxicology. 2013; 7(3):323-37. https://doi.org/10.3109/17435390.2012.658094 PMid:22263604.
29. Geisler-Lee J, Brooks M, Gerfen JR, Wang Q, Fotis C, Sparer AM, Ma X, Berg RH, Geisler M. Reproductive toxicity and life history study of silver nanoparticle effect, uptake and transport in *Arabidopsis thaliana*. Nanomaterials. 2014; 4(2):301-18. https://doi.org/10.3390/nano4020301 PMid:28344224 PMCid: PMC5304678.
30. Kanwar MK, Sun S, Chu X, Zhou J. Impacts of metal and metal oxide nanoparticles on plant growth and productivity. Nanomaterials and plant potential. Springer. 2019; 379-92. https://doi.org/10.1007/978-3-030-05569-1_15
31. Changmei L, Chaoying Z, Junqiang W, Guorong W, Mingxuan T. Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism. Soybean Science. 2002; 21(3):168-71.

32. Jaberzadeh A, Moaveni P, Moghadam HR, Zahedi H. Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. Not Bot Horti Agrobo. 2013; 41(1):201-7. https://doi.org/10.15835/nbha4119093

33. Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea- Torresdey JL. Toxic effects of copper-based nanoparticles or compounds to lettuce *(Lactuca sativa*) and alfalfa (*Medicago sativa*). Environ Sci Process Impacts. 2015; 7(1):177-85. https://doi.org/10.1039/C4EM00551A PMid:25474419 PMCid: PMC4326043.