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# Response of metal-oxide nanoparticles on silique characters in mustard (*Brassica Nigra* L.) varieties

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## Abstract

Mustard (*Brassica nigra* L.) is an important oil crop of India. There has been an expansion of acreage as a result of "Second Green Revolution" brought by the high yielding varieties of the oilseed. Applications of nanotechnology are novel in the field of biotechnology and agriculture. Metal-oxide nanoparticles (NPs) have unique physicochemical properties and possess the potential to boost the plant metabolism. Increasing demand of mustard provokes the research to estimate the feasibility of effective dose NPs for increasing yield potential along with quality production of mustard. Seeds of two mustard varieties namely Shyam 101 and Super teja were soaked for 24 hrs with NPs of iron-oxide, copper-oxide, and zinc-oxide (100, 200, 300, 400, and 500ppm). These soaked seeds were sown in pots and were kept in net house. The results revealed that lower doses of NPs stimulated seed germination in both the varieties whereas higher concentrations of copper-oxide NPs and zinc-oxide NPs strongly inhibited the germination. All the metal-oxide NPs enhanced plant height at 400ppm (at 20 days after sowing) and number of branches at 400ppm (at 40 days after sowing), in both the varieties. The maximum number of silique per plant and the maximum silique length were exhibited by copper-oxide NPs at 400ppm. Higher concentrations of iron-oxide NPs (400ppm) and copper-oxide NPs (300ppm) displayed superior responses for test weight (g) and harvest index (%) respectively.

**Keywords:** metal oxides, nanoparticles, mustard, *Brassica nigra* L.

## Introduction

Nanotechnology holds vast potential in agriculture and allied sciences. Nanotechnology provides an effective tool synthesizing and incorporating nanoparticles(NP) that could augment existing functions, improvising biochemical properties and targeting specific genes manipulation and expression in the specific cells of the plants (Galbriath *et al*., 2007; Cossins *et al*., 2014) [4, 3]. NPs are known to stimulate plants growth and activation of metabolic processes in both plant and animals. Effects of NPs on plants can be beneficial (improving seedling growth and development) or non-beneficial (to prevent root growth) (Zhu *et al*., 2008) [19]. NPs are determined by their chemical composition, size, surface covering, reactivity and most importantly the dose at which they are effective (Kumari *et al*., 2011) [8]. The impact of natural and engineered NPs on higher plants and their beneficial and harmful effects in different plant systems at the physiological, biochemical and genetic levels has recently been examined and documented in the literature (Giraldo *et al*., 2014; Gautam *et al*., 2016a, 2016b; Mishra *et al*., 2016; Rajoriya *et al*., 2016; Shukla *et al*., 2016) [7, 5, 15, 10]. Agricultural production needs to maximize on sustainable basis to meet the continuously increasing food demand of rapidly growing population. Global warming may lead to increased probability of drought and temperature stresses. Continuously increasing use of agro-chemical is threatening human health and environment. Producing more and quality food from diminishing land and water while sustaining agricultural resources base in an environment friendly way is a formidable challenge of this century. To increase productivities in a resource efficient way agriculture needs to be reinforced and revitalized with innovating science-based technologies. (Hafeez *et al*., 2015) [8]. In recent decades nanotechnology products have been intensively applied in agriculture. Nanoparticles are known as a stimulating agent for plant growth and the activation of metabolic processes in plant and animal organisms. Nano-technology as a novel technology has potential to solve many problems in different fields of science and industry and has found its position and functions in agriculture. Nanotechnology has various functions in all stages from production, processing storage, packing and transportation of agricultural products (Scott & Chen *et al.*, 2003) [13]. Brassica species are wild and also grown as food and fodder crops. Brassica nigra is commonly grown for oil extraction, animal cake production, and green manure. It is considered tolerant to heavy metals (Angelova and Ivanov *et al.*, 2009) [2] and also known as metal accumulators and potential phytoextraction (Van Ginneken *et al*., 2007) [18]. Based on resistively and tolerance toward nanoparticles, B. nigra could be considered as a model plant to study reaction mechanisms of metallic nanoparticles on plant growth. Mustard (*Brassica nigra* L.) is an important food oil crop in the semi-arid tropics covering Asia, Africa, Southern Europe, Central and Southern America. This is mainly due to low yield potential of mustard seed under irrigation (Sharma *et al*., 2012) [15]. The present investigation is aimed to reveal physiological action of different metal-oxides-nanoparticles on silique characters in mustard (*Brassica nigra* L.) varieties.

## Material and Methods

### Chemical synthesis of iron-oxide NPs (FeONPs)

Nano-crystalline FeONPs with size ranging from 30-60nm were chemically synthesized following the aqueous solution reduction method with sodium hydroxide as a reducing agent (Mascolo *et al*., 2013). $FeSO_4.7H_2O$, $FeCl_3$ and NaOH were purchased from Merck. For preparing 0.3M of $FeSO_4.7H_2O$, 4.15g of $FeSO_4.7H_2O$ was dissolved in 50ml of deionized water and stirred for 10min in 100 ml flask. In another beaker, 4.85g of $FeCl_3$ was dissolved in 50 ml of deionized water to make 0.6M solution of $FeCl_3$. For preparing 2M NaOH, 8g of NaOH was dissolved in 100ml of deionized water in 500ml flask and stirred continuously. For the synthesis of FeONPs, 50ml each of $FeSO_4.7H_2O$ and $FeCl_3$ was mixed together in a 200ml beaker on magnetic stirrer. NaOH was added drop by drop into the beaker (at an average rate of one drop per second) to reduce the solution till the pH of 10+0.5 was obtained. A dark black precipitate confirmed the formation of FeONPs. Precipitate was removed from supernatant by centrifugation at 12,000rpm for 15min and repeatedly washed with absolute ethanol.

### Copper-Oxide II NPs (CuONPs) and Zinc-Oxide II NPs (ZnONPs)

CuONPs and ZnONPs were procured from Sigma Aldrich. The size of CuONPs were<50nm particles size (TEM), molecular weight 79.55, (TEM, surface area 29 $m^2/g$, from nano power) and the size of ZnONPs were<110nm particles size (DLS), <35nm avg. part. Size (APS) 40 weight % in butyl acetate.

### Mustard Seed Treatment

Mustard seeds of two varieties namely Shayam 101 and Superteja were soaked overnight in water dispersed with varying amount of (100ppm, 200ppm, 300ppm, 400ppm and 500ppm) FeONPs, CuONPs and ZnONPs, respectively along with control (distilled water) on an orbital shaker. After being removed from the soaking, the treated seeds were directly sown in plastic pots (20 x 25 cm) containing 1.0 kg of sand and was kept inside poly house under natural light. After germination, plants were thinned to one healthy plant per pot. The pots were irrigated with tap water throughout the period of investigation. The silique were harvested from three randomly selected and tagged plants in each treatment and were counted and expressed in number, measured in centimeter and 100 seeds of mustard were used to calculate seed test weight.

### Harvest index:

Harvest index of the plants from each pot was recorded by using the formula given below

$$\text{Harvest index} = \frac{\text{Economical yield}}{\text{Biological yield}}$$

## Results

### Number of silique/plants

The maximum number of silique/plant was observed in Shyam 101 with the application of 200ppm each of FeONPs (8.3), CuONPs (9.1) and ZnONPs (9.0), whereas the minimum was observed at 500ppm each of FeONPs (7.6), CuONPs (7.0) and ZnONPs (4.3). Similarly, maximum no. of silique was observed in Superteja treated with 400ppm each of FeONPs (9.6), CuONPs (10.6) and ZnONPs (9.3), whereas the minimum was observed of 500ppm each of FeONPs (7.0), CuONPs (6.6) and ZnONPs (6.6) (Table1, Fig1).

**Table 1**: Effect of different concentrations of metal-oxide-nanoparticles on average no. of silique in mustard varieties (Shyam 101 and Superteja)/plant.

| Treatments | Average no. of silique/ Plant | | | | | |
|---|---|---|---|---|---|---|
| | Shyam 101 | | | Superteja | | |
| | FeONPs | CuONPs | ZnONPs | FeONPs | CuONPs | ZnONPs |
| Control | 8.3 | 9.0 | 7.0 | 7.0 | 9.0 | 8.0 |
| 100 ppm | 7.6 | 7.6 | 8.6 | 8.3 | 6.6 | 8.6 |
| 200 ppm | 8.3 | 7.0 | 9.0 | 9.6 | 8.0 | 9.3 |
| 300 ppm | 7.6 | 7.6 | 7.6 | 7.6 | 9.3 | 6.6 |
| 40Oppm | 8.0 | 8.6 | 5.0 | 7.0 | 10.6 | 6.3 |
| 500 ppm | 7.6 | 7.3 | 4.3 | 8.6 | 7.6 | 6.6 |
| Gen.Mean | 7.9 | 7.8 | 6.9 | 8.0 | 8.5 | 7.6 |
| C.V. | 8.3 | 5.9 | 15.5 | 14.6 | 9.9 | 21.2 |
| S.Em | 0.3 | 0.2 | 0.6 | 0.6 | 0.4 | 0.9 |
| C.D. 5% | _ | 0.8 | 1.9 | _ | 1.5 | _ |

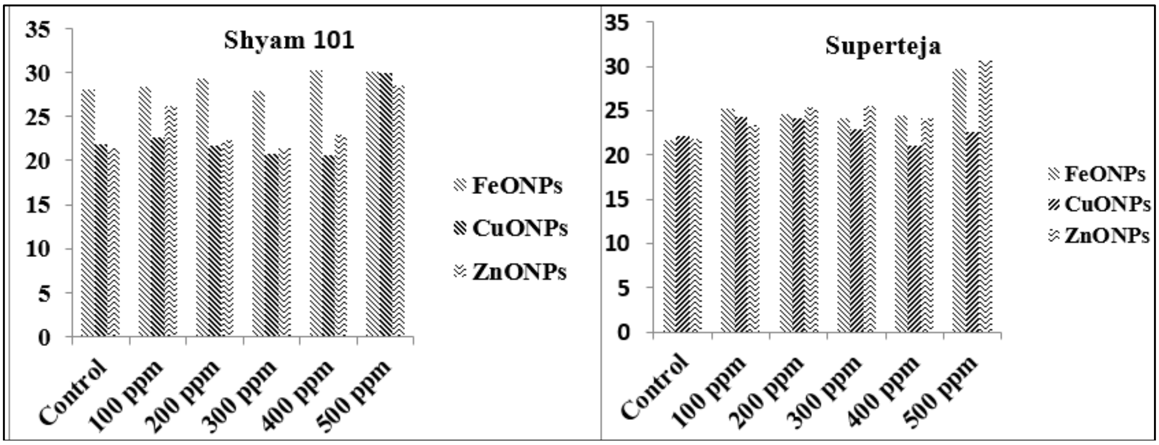

**Fig 1:** Effect of different concentrations of metal-oxide-nanoparticles on average no. of silique in mustard varieties (Shayam 101 and Superteja)/plant

### Silique Length (cm)

The maximum length of silique(cm) was observed in Shayam 101 with the application of 400ppm each of FeONPs(6.8), CuONPs(7.2) and ZnONPs(5.3), whereas the minimum was observed at 500ppm each of FeONPs(5.5), CuONPs(5.2) and ZnONPs(3.1) and maximum was observed in Superteja at 400ppm each of FeONPs(6.5), CuONPs(6.6) and ZnONPs(7.0), whereas the minimum was observed at 500ppm each of FeONPs(5.4), CuONPs(5.4) and ZnONPs(4.1) (Table 2, Fig 2)

**Table 2:** Effect of different concentrations of metal-oxide-nanoparticles on average of silique length (cm) in mustard varieties (Shayam 101 and Superteja).

| **Treatments** | **Average silique length (cm)** | | | | | |
|---|---|---|---|---|---|---|
| | **Shyam 101** | | | **Superteja** | | |
| | **FeONPs** | **CuONPs** | **ZnONPs** | **FeONPs** | **CuONPs** | **ZnONPs** |
| Control | 6.8 | 7.2 | 5.3 | 6.5 | 6.5 | 7.0 |
| 100 ppm | 6.5 | 6.1 | 5.1 | 5.8 | 5.8 | 7.0 |
| 200 ppm | 5.6 | 5.7 | 5.3 | 6.2 | 6.3 | 5.4 |
| 300 ppm | 5.5 | 5.2 | 3.9 | 5.7 | 5.4 | 4.4 |
| 400 ppm | 5.8 | 5.7 | 3.6 | 5.4 | 6.6 | 4.1 |
| 500 ppm | 5.7 | 6.4 | 3.1 | 5.4 | 5.4 | 4.5 |
| Gen. Mean | 6.0 | 6.0 | 4.4 | 5.8 | 6.0 | 5.4 |
| C.V. | 8.2 | 7.8 | 4.6 | 5.7 | 5.0 | 5.9 |
| S. Em | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| C.D. 5% | 0.8 | 0.8 | 0.3 | 0.5 | 0.5 | 0.5 |

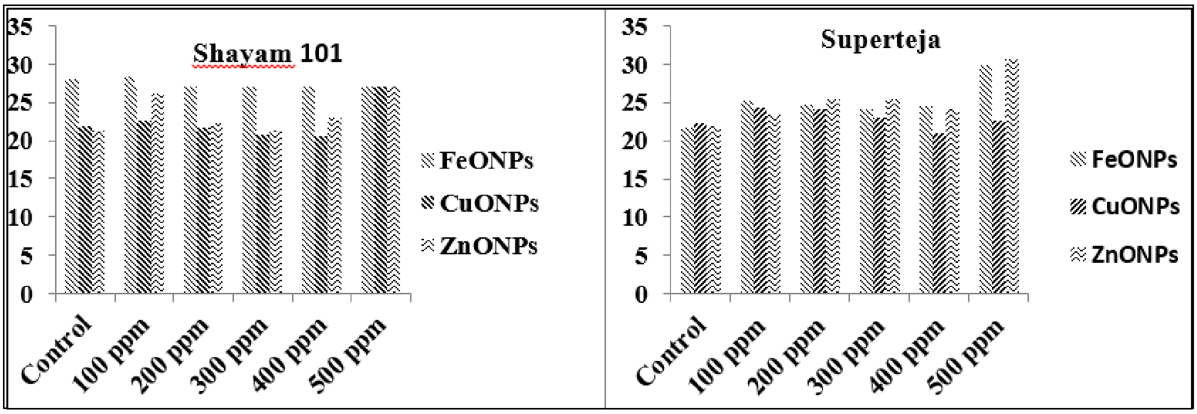

**Fig 2:** Effect of different concentrations of metal-oxide-nanoparticles on average of silique length (cm) in mustard varieties (Shayam 101 and Superteja)/plant.

### Test weight (g)

The maximum test weight was observed in Shyam 101 with the application of 300ppm each of FeONPs (1.9), CuONPs (1.9) and ZnONPs (1.9), whereas the minimum was observed of 500ppm each of FeONPs (1.3), CuONPs (1.3) and ZnONPs (1.4). Similarly, maximum was observed in Superteja at 400ppm each of FeONPs (1.8), CuONPs (1.7) and ZnONPs (1.4), whereas the minimum was observed at 500ppm each of FeONPs (1.3), CuONPs (1.3) and ZnONPs (1.2) (Table3, Fig3)

**Table 3:** Effect of different concentrations of metal-oxide-nanoparticles on test weight in mustard varieties (Shayam 101 and Superteja).

| **Treatments** | **Test Weight (g)** | | | | | |
|---|---|---|---|---|---|---|
| | **Shyam 101** | | | **Superteja** | | |
| | **FeONPs** | **CuONPs** | **ZnONPs** | **FeONPs** | **CuONPs** | **ZnONPs** |
| Control | 6.8 | 7.2 | 5.3 | 6.5 | 6.5 | 7.0 |
| 100 ppm | 6.5 | 6.1 | 5.1 | 5.8 | 5.8 | 7.0 |
| 200 ppm | 5.6 | 5.7 | 5.3 | 6.2 | 6.3 | 5.4 |
| 300 ppm | 5.5 | 5.2 | 3.9 | 5.7 | 5.4 | 4.4 |
| 400 ppm | 5.8 | 5.7 | 3.6 | 5.4 | 6.6 | 4.1 |
| 500 ppm | 5.7 | 6.4 | 3.1 | 5.4 | 5.4 | 4.5 |
| Gen. Mean | 6.0 | 6.0 | 4.4 | 5.8 | 6.0 | 5.4 |
| C.V. | 8.2 | 7.8 | 4.6 | 5.7 | 5.0 | 5.9 |
| S. Em. | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| C.D. 5% | 0.8 | 0.8 | 0.3 | 0.5 | 0.5 | 0.5 |

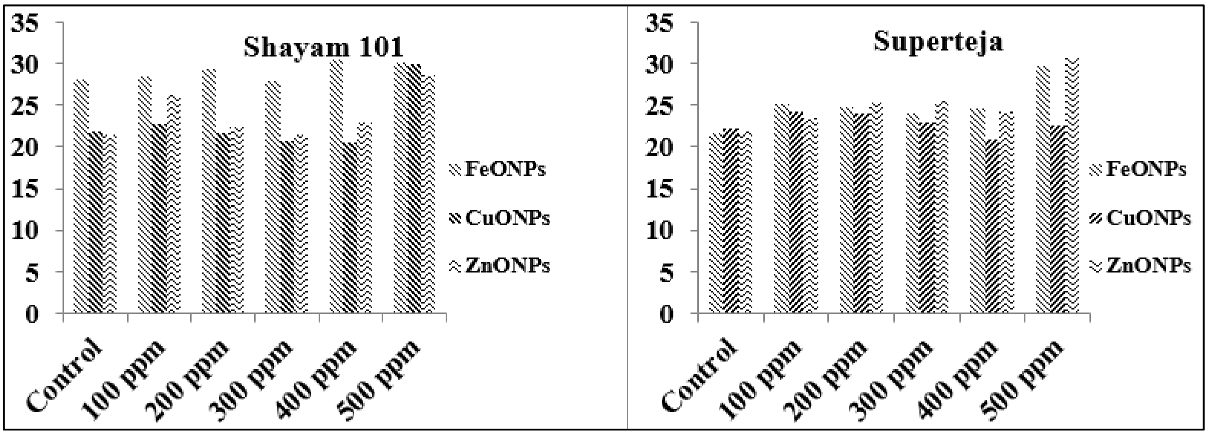

**Fig 3:** Effect of different concentrations of metal-oxide-nanoparticles on test weight in mustard varieties (Shayam 101 and Superteja)/plant.

## Harvest index (%)

The maximum harvest index was observed in Shayam 101 with the application of 500 ppm each of FeONPs(34.4%), CuONPs(29.9%) and ZnONPs(28.6%), whereas the minimum was observed of 300ppm each of FeONPs(27.9%), CuONPs(20.6%) and ZnONPs(21.4%) and maximum was observed in Superteja of 500ppm FeONPs(29.8%), CuONPs(24.3%) and ZnONPs(30.6%), whereas the minimum was observed at 400ppm each of FeONPs(21.7%), CuONPs(21.0%) and ZnONPs(21.8%) (Table4,Fig4)

**Table 4:** Effect of different concentrations of metal-oxide-nanoparticles on harvest index in mustard varieties (Shayam 101 and Superteja)/plant

| **Treatments** | **harvest Index %** | | | | | |
|---|---|---|---|---|---|---|
| | **Shyam 101** | | | **Superteja** | | |
| | **FeONPs** | **CuONPs** | **ZnONPs** | **FeONPs** | **CuONPs** | **ZnONPs** |
| Control | 28.0 | 21.8 | 21.4 | 21.7 | 22.2 | 21.8 |
| 100 ppm | 28.4 | 22.7 | 26.2 | 25.2 | 22.6 | 23.4 |
| 200 ppm | 29.3 | 21.7 | 22.3 | 24.7 | 24.1 | 25.4 |
| 300 ppm | 27.9 | 20.8 | 21.4 | 24.5 | 23.0 | 25.5 |
| 400 ppm | 30.1 | 20.6 | 22.9 | 24.1 | 21.0 | 24.2 |
| 500 ppm | 30.4 | 29.9 | 28.6 | 29.8 | 24.3 | 30.6 |
| Gen. Mean | 29.0 | 22.9 | 23.8 | 25.0 | 22.9 | 25.2 |
| C.V. | 3.1 | 4.2 | 4.6 | 7.9 | 7.7 | 3.5 |
| S. Em. | 0.5 | 0.5 | 0.6 | 1.1 | 1.0 | 0.5 |
| C.D. 5% | 1.6 | 1.7 | | 3.5 | _ | 1.5 |

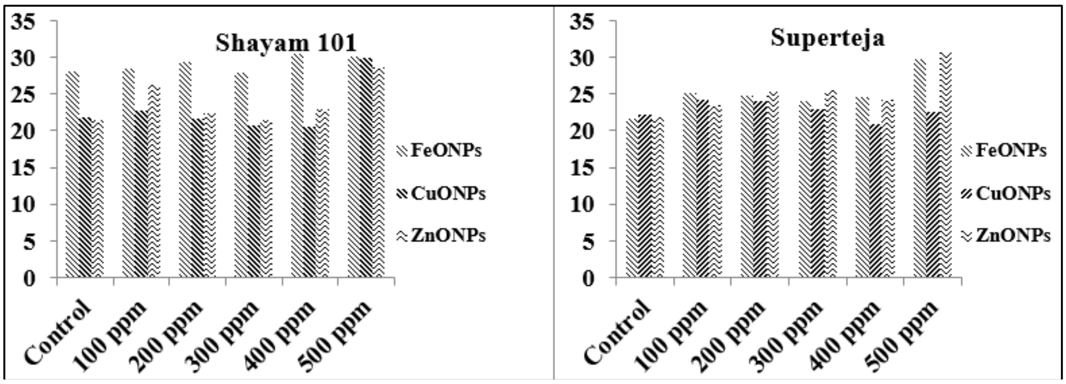

**Fig 4:** Effect of different concentrations of metal-oxide-nanoparticles on harvest index in mustard varieties (Shayam 101 and Superteja)/plant.

## Discussion

NPs are known as a stimulating agent for plant growth. Engineered NPs are able to enter into plants cells and leaves, and can also transport DNA and chemicals into plants cells. This area of research offers new possibilities in plant biotechnology to target specific genes manipulation and expression in the specific cells of the plants (Galbriath *et al*., 2007; Torney *et al*., 2007) [4, 18]. Effect of NPs on germination depends on concentrations of NPs and varies from plants to plants. Different conc. of ZnONPs were applied on cucumber, mustard and tomato, and found that only cucumber and mustard seed germination was enhanced (Tarafdar *et al*., 2013) [17]. ZnONPs increase plant growth and development. Lower conc. of ZnONPs exhibited beneficial effect on seed germination in onion. However, higher dose of ZnONPs impaired seed germination. NPs absorbed by different plant species can produce positive and negative effects (Raskar *et al*., 2014) [12]. Effect of CuONPs (<50nm) into the root system was responsible for the toxicity. A parallel experiment was also carried out to know the effect of copper sulphate solution on seed germination, above 200ppm Cu; it restricted the germination of seeds, because of high salinity (Adhikari, 2012) [1]. Fe is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang *et al*., 2011; Samaranayke *et al.*, 2012) [21, 13] .

## Conclusion

Different concentrations of FeONPs, CuONPs and ZnONPs responded in various ways exhibiting significant biological effects on silique characters and yield in mustard. The results revealed that the maximum number of silique/plant and the maximum silique length were exhibited by copper oxide NPs at 400 ppm. Higher concentrations of iron oxide NPs (400 ppm) and copper oxide NPs (300 ppm) displayed superior responses for test weight (g) and harvest index (%), respectively.

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