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Original article

Green synthesis of zinc nanoparticles and their effects on growth and yield of *Pisum sativum*

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ABSTRACT

Zinc is one of the crucial micronutrients required for the sustenance of growth and metabolism in plants. Zinc deficiency results in growth inhibition and chlorosis in plants along with low production of carbohydrates, proteins, and chlorophyll. The utilization of zinc nanoparticles (ZnNPs) is an effective strategy to enhance growth in plants. The present investigation was carried out to determine the effect of green synthesized ZnNPs on the growth and yield of *Pisum sativum* L. Two pea varieties were raised in pots treated with various levels of ZnNPs. Zinc nanoparticles escalated the growth and yield of pea plants. Maximum growth and yield were obtained at 400 and 600 ppm as compared to control and zinc sulphated treated plants. In conclusion green synthesized zinc nanoparticles can enhance crop pea plant's growth and productivity. More investigations are suggested under variable agro-climatic zones for declaration of the best application rate between 400 and 600 ppm for different crops.

1. Introduction

Zinc is a crucial micronutrient needed for the normal development and growth of both plants and humans (Hafeez, 2013) but zinc is not available to plants as its deficiency in soil is extensive worldwide (Cakmak, 2008; White and Broadley, 2009). Its deficiency reduces the yield of major crops. It causes a reduction in plant biomass, stunted growth and chlorosis of young leaves (Broadley et al., 2007). Due to these reasons, zinc is getting much more attention worldwide (Cakmak, 2008). Different approaches have been practised for the past few decades to overcome this problem (Pfeiffer and McClafferty, 2007). Conventional fertilizers which are being used have many adverse effects like groundwater pollution as a result of leaching, chemical burn to crops, nutrient imbalance in the root zone, acidification of the soil and mineral

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depletion of the soil. It can also increase the abnormal development of some natural weeds or micro-organisms which can disturb the agro-ecosystems. So, there is a need to pay attention to introduce some advanced technology such as "Nanotechnology" to overcome the nutrient deficiency. Nanotechnology is defined as the applied field of science that deals with the production, characterization and application of structures, devices, materials and systems at the nanoscale (Dhoke et al., 2013).

There is an opportunity for the revolution of agricultural systems through nanotechnology (Dimkpa and Bindraban, 2016). For the sophisticated delivery structure for agrochemicals, nano fertilizers should be used which are ecofriendly and has an easy way of delivery. Nano fertilizers are more operative as compared to conventional fertilizers due to their high surface area to volume ratio and their behaviour could encourage the crops for effective nutrient uptake. There is an increase in soil fertility and plant health restoration, applied fertilizers effectiveness, environment pollution dropping and degradation of an agricultural ecosystem with the help of nanoclays, condensed nanoparticles and zeolites (Manjunatha et al., 2016). Zinc nanoparticles can also be used as nanofertilizer in the agricultural field. But applications of nanoparticles depend upon the method used to synthesize particular nanoparticles as well as properties related to their size (Prakasham et al., 2014).

There are two main synthetic strategies for the production of nanoparticles, one of them is the top-down approach and the other is a bottom-up approach. A top-down approach is used to synthesize the nanomaterials by nanomachining, fragmentation (nanomilling and spark erosion) and lithography. While, a bottom-up approach includes the physical, chemical and biological methods. Various biological systems i.e. yeast, fungi and bacteria have been used for the biogenic synthesis of nanoparticles (Alagumuthu and Kirubha, 2012). Great attention is paid to the synthesis of metallic nanoparticles by using organisms. When microorganisms are used for the synthesis of nanoparticles it requires many intricate processes like intracellular synthesis, cell culture maintenance and multiple purification steps. Therefore, it is somewhat difficult to biosynthesize the nanoparticles by using microorganisms. On the other hand, plants are the most suitable among these organisms for the large-scale synthesis of nanoparticles. The resulting nanoparticles are stable having variations in their size and shape as compared to those nanoparticles which are produced by other organisms (Ramesh et al., 2014). It is the most preferable method for the synthesis of zinc oxide nanoparticles due to the eco-friendliness and simplicity of the biological system (Gunalan et al., 2012). The process in which nanoparticles are synthesized with plant-based material is termed green synthesis (Mason et al., 2012).

Vegetables are desirable for good health because they are a significant source of micronutrients and act as nutritional powerhouses. In our diet, assortment, nutritional quality and flavour are added by vegetables. For the agricultural economy, the productive and commercial engines are vegetables. Pea (*Pisum sativum* L.) is a leguminous crop and belongs to the family Fabaceae. After soybean and common beans, the world's third most noteworthy legume grain is pea. Due to the starch content, protein and other nutrients of pulses including pea are the main components of human beings. The consumption of peas is much more attractive not due to their nutritional value (Timmerman-Vaughan et al., 2005). From a genetic and management point of view, lots of efforts have been required regarding the improvement of peas yield (Chisti et al., 2018). The current research aims to check the effects of different concentrations of green synthesized zinc nanoparticles on the growth and yield of the Pea plant. It is hypothesized that green synthesized zinc nanoparticles have the potential to improve pea plant's growth and yield.

2. Materials and methods

2.1. Materials and chemicals

Hybrid seeds of two Pea (*Pisum sativum* L.) varieties, Meteor and Sprinter, were purchased from Punjab Seed Corporation, Lahore. Systematic grades of the following chemicals were used in the current experiment; Zinc Sulphate, Sodium Carbonate, Oxalic acid, 2,6-dichlorophenol indophenols (DCPIP), Follin's Ciocalteu, Sodium Potassium Tartrate, Oxalic Acid, Ascorbic Acid, EDTA, Copper Sulphate, Acetone, Anthrone reagent, Bovine Serum Albumin, Methanol, Sodium Hydroxide, Phenapthalein, Methyl orange indicator and Sulphuric acid (H_2SO_4).

2.2. Green synthesis of zinc nanoparticles

Clove buds (plant material) bought from the native market were transformed into powder by using an electric grinder. Electric balance was used to weigh 20 g of clove buds precisely and taken in a beaker along with 100 mL of distilled water. The extract was prepared in a microwave oven under the following conditions i.e., 1000 W for 130–150 s. The extract was cooled at room temperature (20–22 °C) and strained. Zinc nanoparticles were made with the reaction of 10 mL plant extract and 10 mL of zinc sulphate solution. The formation of zinc nanoparticles was examined after four hours. Solution of ZnNPs solution was taken in Eppendorf's tube for centrifugation at 10,000 rpm and 10 min to separate the nanoparticles in form of pallets. This process of centrifugation was repeated 3–4 times with distilled water for purification. Then, prepared NPs were desiccated at 50 °C for four to five hrs. and stored in the refrigerator at 4 ℃ for further use (Javad et al., 2017).

2.3. Characterization of zinc nanoparticles

Green synthesized ZnNPs were characterized by using following techniques.

2.3.1. Ultraviolet spectroscopy

Colour change of the solution was observed for confirmation of ZnNPs synthesis. Along with this, UV–Vis spectrophotometry was also used. The absorption spectrum of the prepared sample was taken within 300–800 nm range at (BMS UV-2600) spectrophotometer.

2.3.2. Scanning electron microscopy (SEM)

For morphological analysis of zinc nanoparticles, a scanning electron microscope (EVO-LS10) was used. The dried sample was exposed to the beam of electrons which provided the image and size of ZnNPs.

2.3.3. Fourier transform infrared (FTIR) spectroscopy

For FTIR inspection, the prepared sample was required to examine capping agents on the surface of ZnNPs. In this method, dried powder of ZnNPs was required. The FTIR analysis of dried powder was carried out by (Shimadzu: IRTracer-100) under the following conditions reduction of total reflection mode, four cm^{-1} resolution with 4000–400 cm^{-1} spectral range.

2.4. Effect of zinc nanoparticles on pea plant

The pot experiment of the present work was completed in Botanical Garden (74°21-00-E, 31°35-00-N), Quaid-e-Azam Campus, University of Punjab, Lahore from October 2019 to December 2019. The experiment was carried out in a wire-netting house to hinder the animal's violence. Earthen pots were filled with a mixture of sandy and loamy soil in 1:3 ratio along with compost and animals' manure to make it nutrient enriching. An experiment was done in three replicates using RCBD (Randomized Complete Block Design). Seeds were soaked in clean tap water for 24 h before sowing. The experiment was maintained using normal agricultural practices. The stock solution of Zinc NPs was made and diluted further in different ppm concentrations to observe the consequences of different ZnNPs concentrations on Pea varieties during the whole season of 2019. Different concentrations of zinc NPs used in the current research are control, $ZnSO_4$, ZnNPs-100, ZnNPs-200, ZnNPs-400, ZnNPs-600, ZnNPs-800 and ZnNPs-1000.

During the experimental season, zinc nanoparticle solution was applied four times at regular intervals on the pea plant as a soil drench. Zinc nanoparticles were applied in 100 mL as soil drench using above mentioned concentrations. Control was irrigated only with tap water taken as a negative control. Positive control was provided with 1000 ppm solution of $ZnSO_4$ (bulk material).

2.5. Measurement of parameters

2.5.1. Morphological parameters

Three pots per treatment were taken at 60 days after sowing. The plants were removed with their roots carefully, washed thoroughly, packed in labelled bags and carried to the laboratory for measurement of the following parameters: root length (cm), shoot length (cm), number of leaves, leaf area (cm^2), number of leaflets, leaflet area, number of nodules plant^{-1} and number of stipules plant^{-1}. By using the Carleton and Foote (Carleton and Foote, 1965) the leaf area was calculated as;

$$\text{Leaf area} = \text{Leaf length} \times \text{width} \times 0.75\,(\text{correction factor})$$

2.5.2. Biomass assessment

Biomass assessment was done after the morphological parameter measurement including the fresh weight of plants(g), root fresh weight (g), shoot fresh weight (g), leaves fresh weight (g), fresh weight of seeds (g), fresh weight of pods (g) and total fresh weight (g) were also determined. The plants were packed in labelled paper bags and placed in a drying oven (Wiseven, Model WOF-105, Korea) at 70 °C for 72 h and dry weights of their root, shoot, leaves, pods and seeds were measured simply by using the electric balance (Sartorius GMBH, Type 1216MP 6E, Gottingen, Germany).

2.6. Yield parameters

Yield parameters as the number of flowers plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, number of seeds plant^{-1} and pod length were recorded.

2.7. Statistical analysis

The mean and standard error of data obtained from the pot experiment were calculated and for this purpose, the Software package SPSS (version 20) was employed (Steel et al., 1997).

3. Results

The present experiment was carried out to find out the effect of green synthesized zinc nanoparticles on the growth and productivity of pea (var. Meteor and Sprinter). Different concentrations of zinc nanoparticles e.g., ZnNP-100 ppm, ZnNP-200 ppm, ZnNP-400 ppm, ZnNP-600 ppm, ZnNP-800 ppm and ZnNP-1000 ppm along with $ZnSO_4$ were applied to pea varieties by using the soil drench application.

3.1. Characterization of zinc nanoparticles

3.1.1. Ultraviolet spectroscopy

Characterization of zinc nanoparticles was done by using the absorption spectra obtained from Ultraviolet spectroscopy. The exposure of ZnNPs to the UV radiations showed a peak at 374 nm^{-1}, which makes sure that nanoparticles were present since the zinc nanoparticles lie between the ranges of 300 to 400 nm^{-1}. The peak along with the absorption spectrum is shown in Fig. 1. The nanoparticles synthesized from clove buds were white.

3.2. Study of morphological parameters

Zinc nanoparticles exhibited a significant effect on morphological characters of *Pisum sativum* L. (Pea) as shown in Plate 3.1(a & b).

3.2.1. Number of leaves and leaflets

The number of leaves and leaflets were recorded for control and treated plants grown under the field conditions at 60 DAS and is shown in Table 1. The number of leaves and leaflets was increased as the concentrations of zinc nanoparticles were increased. The maximum number of leaves and leaflets were observed in ZnNPs-400 (14.67, 48.33) and 600 ppm (12.67, 43.67) of variety Meteor while the minimum in ZnNPs-800 (4.67, 25.67) and 1000 ppm (3.67, 22.33). The same pattern was observed in var. Sprinter that the maximum number of leaves and leaflets was found in ZnNP-400 (14.33, 46.67) and ZnNP-600 ppm (11.67, 40.67) while the minimum number of both leaves and leaflets was observed in ZnNP-800 (4.33, 24.67) and ZnNP-1000 ppm (3.33, 20.33) treated pea plants The number of leaves and leaflets were increased or decreased in the following manner in both variety of pea of zinc nanoparticles ZnNPs-400 > ZnNPs-600 > ZnNPs-200 > ZnNPs-100 > ZnSO4 > control > ZnNPs-800 > ZnNPs-1000.

Overall maximum percentage increase of leaves and leaflets was 67.49% and 67.5% at ZnNPs-400 ppm of var. Meteor while minimum −34.21% and −34.11% at ZnNPs-1000 ppm of var. Sprinter.

3.2.2. Area of leaf and leaflet

Zinc nanoparticles had a better effect on leaf and leaflet area as compared to zinc sulphate. But a very high level of zinc nanoparticles causes toxicity in plants. The width and length of both leaf and leaflet showed marked variation with increasing levels of zinc nanoparticles in both varieties of pea which affect the area directly.

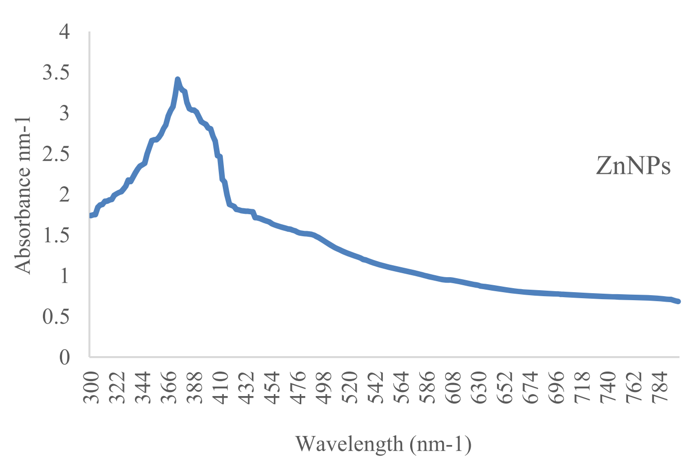

Fig. 1. UV–Visible spectrum of green synthesized ZnNPs showing a peak at 374 nm^{-1}.

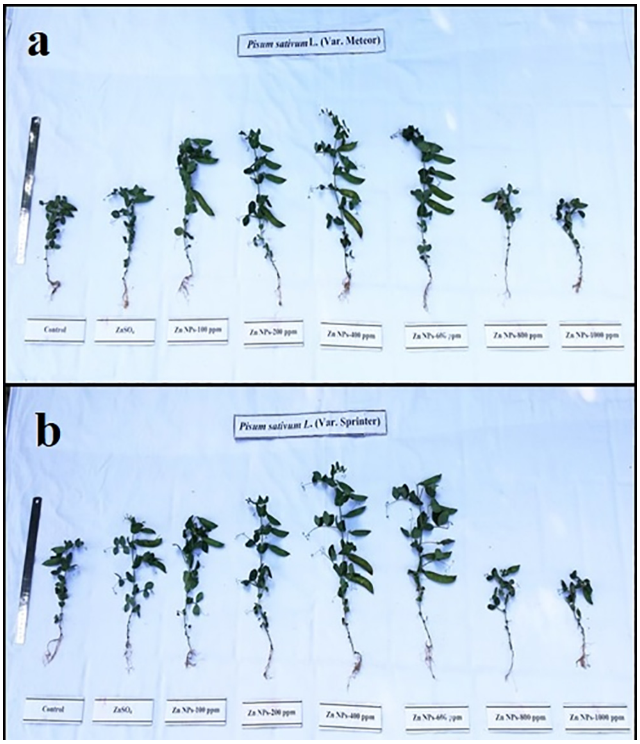

Plate 3.1. Effect of different concentrations of Zinc Nanoparticles on yield of *Pisum sativum* L. Var. (a) Meteor and (b) Sprinter) harvested at 60 DAS by using a soil drench.

The area was significantly increased with an increase in zinc nanoparticles concentration but at much highest level onward to 600 ppm, it causes toxicity and had a negative impact on leaf and leaflet area. At 60 DAS, maximum leaf and leaflet area were observed in plants treated with ZnNPs-400 ppm (67.17, 11.83 cm^2) and ZnNPs-600 ppm(61.33, 10.73 cm^2) while minimum leaf and leaflet area was found in ZnNPs-800 ppm (35.88, 4.94 cm^2) and ZnNPs-1000 ppm (30.22, 3.85 cm^2) treated plant under soil drench application in var. Meteor. In variety Sprinter, the pattern for increase or decrease in leaf and leaflet area was the same as in var. Meteor with slight differences. It was concluded that the result of leaf and leaflet area was better in var. meteor on the other hand the results were also good with slight differences.

3.2.3. Shoot length

Eight different concentrations of ZnNPs along with zinc sulphate were used during the present experiment given as soil drench to two varieties of pea i.e., Meteor and Sprinter. The maximum plant height (4142, 39.67 cm) was recorded at 400 and 600 ppm respectively while minimum plant height (25.39, 23.75 cm) was recorded at 800 and 1000 ppm concentration of zinc nanoparticles applied as a soil drench in a variety meteor. In this variety, at ZnNPs-400 ppm plant height (cm) was increased 42.86% from zinc sulphate and 52.86% from control plants. The percentage increase in shoot length for (var. Meteor) plants treated other concentrations i.e. $ZnSO_4$, ZnNPs-100, ZnNPs-200, ZnNPs-800 and ZnNPs-1000 ppm at 60 DAS were recorded as follows 13.98%, 22.85%, 33.88%, −14.04% and −19.57% respectively and the percentage increase of (var. Sprinter) plants treated other concentrations i.e. zinc sulphate, ZnNPs-100, ZnNPs-200, ZnNPs-800 and ZnNPs-1000 ppm were recorded as follows 12.46%, 21.39%, 31.05%, −9.68% and −20.57% respectively. However, at ZnNPs-400 and ZnNPs-600 ppm concentration, plants showed more shoot length at that concentration zinc act as a micronutrient, and it caused toxicity as the concentration increased.

3.2.4. Root length

The effect of ZnNPs had a direct relationship with the root length. As the concentration of ZnNPs increased, the root length increased and vice versa. The data given in Table 1 shows that both varieties meteor and sprinter of ZnNPs cause a marked enhancement in root length.

The data obtained showed the increasing trend in both varieties of Pea. The maximum root lengths were 21.3 cm and 22.37 cm for var. Meteor and var. Sprinter of ZnNPs-400 respectively while the lowest values were 10.17 cm and 8.6 cm for var. Meteor and Sprinter atZnNPs-1000. It showed a gradual increase in root length from

Table 1
Morphological growth parameters of *Pisum sativum* L. (var. Meteor and Sprinter) harvested at 60 DAS by using the soil drench under different zinc nanoparticles concentration.

Varieties of Pea	Treatments	Morphological Growth Parameters								
		No. of Leaves	No. of Leaflets	Leaf Area (cm^2)	Leaflet Area (cm^2)	Shoot Length (cm)	Root Length (cm)	No. of Stipules	No. of Root Nodules	No. of Tendrils
Meteor	Control	5.67f ± 0.3	29.33f ± 0.5	43.74f ± 0.2	6.43f ± 0.6	29.53f ± 0.3	14.49f ± 0.1	7.33f ± 0.5	23.67f ± 0.3	29.33f ± 0.2
	$ZnSO_4$	6.67e ± 0.6	33.33e ± 0.3	47.34e ± 0.7	7.56e ± 0.2	32.78e ± 0.1	16.95e ± 0.4	8.33e ± 0.8	27.67e ± 0.3	33.33e ± 0.4
	ZnNPs-100	8.67d ± 0.2	36.67d ± 0.2	50.51d ± 0.5	8.88d ± 0.3	35.99d ± 0.4	17.59d ± 0.2	9.33d ± 0.2	32.67d ± 0.7	36.67d ± 0.3
	ZnNPs-200	10.67c ± 0.4	39.33c ± 0.6	55.13c ± 0.4	9.84c ± 0.7	36.59c ± 0.2	19.99c ± 0.4	10.33c ± 0.1	36.67c ± 0.2	39.33c ± 0.2
	ZnNPs-400	14.67a ± 0.3	48.33a ± 0.1	67.17a ± 0.1	11.83a ± 0.2	41.42a ± 0.7	26.83a ± 0.3	12.67a ± 0.6	44.67a ± 0.9	48.33a ± 0.3
	ZnNPs-600	12.67b ± 0.31	43.67b ± 0.8	61.33b ± 0.7	10.73b ± 0.1	39.67b ± 0.3	21.45b ± 0.6	11.33b ± 0.4	40.67b ± 0.2	43.67b ± 0.1
	ZnNPs-800	4.67 g ± 0.5	25.67 g ± 0.3	35.88 g ± 0.3	4.94 g ± 0.3	25.39 g ± 0.6	12.47 g ± 0.3	6.33f ± 0.3	17.33 g ± 0.1	25.67 g ± 0.6
	ZnNPs-1000	3.67 h ± 0.9	22.33 h ± 0.2	30.22 h ± 0.2	3.85 h ± 0.4	23.75 h ± 0.3	11.13 h ± 0.7	4.33f ± 0.3	11.67 h ± 0.3	22.33 h ± 0.5
Sprinter	Control	5.33f ± 0.2	27.67f ± 0.7	40.26f ± 0.7	5.19f ± 0.7	27.55f ± 0.1	13.34f ± 0.1	6.67f ± 0.2	20.67f ± 0.3	27.67f ± 0.3
	$ZnSO_4$	6.33e ± 0.7	31.67e ± 0.2	45.45e ± 0.2	7.23e ± 0.9	30.99e ± 0.8	15.56e ± 0.8	7.67e ± 0.1	25.67e ± 0.6	31.67e ± 0.1
	ZnNPs-100	8.33d ± 0.1	34.67d ± 0.6	48.17d ± 0.8	8.08d ± 0.8	33.45d ± 0.2	17.23d ± 0.4	8.67d ± 0.8	30.67d ± 0.9	34.67d ± 0.8
	ZnNPs-200	9.67c ± 0.3	38.33c ± 0.4	54.2c ± 0.2	9.55c ± 0.3	35.11c ± 0.9	18.67c ± 0.9	9.67c ± 0.3	34.67c ± 0.5	38.33c ± 0.4
	ZnNPs-400	14.33a ± 0.2	46.67a ± 0.3	65.27a ± 0.7	11.17a ± 0.5	40.44a ± 0.5	24.27a ± 0.6	11.67a ± 0.2	43.67a ± 0.3	46.67a ± 0.2
	ZnNPs-600	11.67b ± 0.7	40.67b ± 0.5	59.4b ± 0.2	10.47b ± 0.1	38.24b ± 0.3	20.73b ± 0.3	10.67b ± 0.1	39.33b ± 0.2	40.67b ± 0.4
	ZnNPs-800	4.33 g ± 0.9	24.67 g ± 0.1	33.47 g ± 0.3	4.56 g ± 0.3	24.89 g ± 0.6	11.47 g ± 0.3	5.67 g ± 0.5	14.67 g ± 0.1	24.67 g ± 0.7
	ZnNPs-1000	3.33 h ± 0.4	20.33 h ± 0.2	26.33 h ± 0.1	3.73 h ± 0.5	21.89 h ± 0.2	9.43 h ± 0.4	3.33 g ± 0.3	9.67 h ± 0.7	20.33 h ± 0.8

Each treatment mean is sum of the three replicates and ± represents standard error (SE).Within each parameter values not followed by same letter are significantly different at Duncan's multiple range test and P = 0.05; ZnNPs; Zinc Nanoparticles.

ZnNPs-100 to ZnNPs-600 in both varieties and after that concentration, it started to decrease. Among all treatments, 400 ppm showed better results as compared to zinc sulphate and control.

3.2.5. Number of stipules

It was revealed that zinc nanoparticles at different concentrations significantly increased the number of stipules. The number of stipules in both varieties (i.e., Meteor and Sprinter) gradually increased as the concentration increased but at the highest concentration (800 and 1000 ppm) it started to decrease. In var. Mtetor, among the treatments, the application of ZnNPs-400 had a maximum number of stipules (12.67). But after 400 ppm concentration, the stipules number decreased with increasing level of zinc nanoparticles because it started to create toxicity in growing plants and suppressed their growth. The same trend of results was examined in var. Sprinter (Table 1).

3.2.6. Number of root nodules

Zinc nanoparticles had also a positive effect on root nodules and these numbers increased with increasing levels of zinc nanoparticles. Both pea varieties showed the same behaviour in response to different concentrations. In var. Sprinter, a maximum number of root nodules (44.67, 43.67) were recorded at 400 ppm in var. Meteor and Sprinter respectively while the minimum values atZnNPs-1000 ppm. In both varieties, increasing the level of concentration had the same effect on the number of root nodules. But in var. Meteor the concentration 400 ppm had more number as compared to var. Sprinter at same concentration.

3.3. Biomass assessment

3.3.1. Root, shoot and leaves fresh weight

It was evident from the result that ZnNPs application significantly increased the fresh weight of *Pisum sativum* L. (Pea) from 100 to 600 ppm concentration, but the fresh weight started to decrease from 800 ppm. The fresh weight of root, shoot and leaves of pea was highest at 400 ppm ZnNPs treatment. Fresh matter production was lowest at 1000 ppm ZnNPs treated plant. The highest fresh weight was observed at 400 ppm in var. Meteor while lowest observed at 1000 ppm in var. Sprinter. The percentage increase recorded was maximum at 400 and 600 ppm concentration of zinc nanoparticles for both varieties; 186.25 and 161.66% respectively for root fresh weight, 29.43 and 26.95% respectively for shoot fresh weight and 64.29% and 54.72% for leaves fresh weight of var. Meteor while 172.46% and 150% for root, 27.81% and 24.36% for the shoot and 62.62% and 52.71% for leaves fresh weight of var. Sprinter during the present investigation.

The comparison of variety Meteor and Sprinter during the entire experiment showed that the percentage increase in the root, shoot and leaves fresh weight were less when the application of $ZnSO_4$was applied as compared 100–600 ppm of zinc nanoparticles concentration. Fig. 2 shows the pattern of percentage increase in root shoot and leaves for both varieties i.e., Meteor and Sprinter.

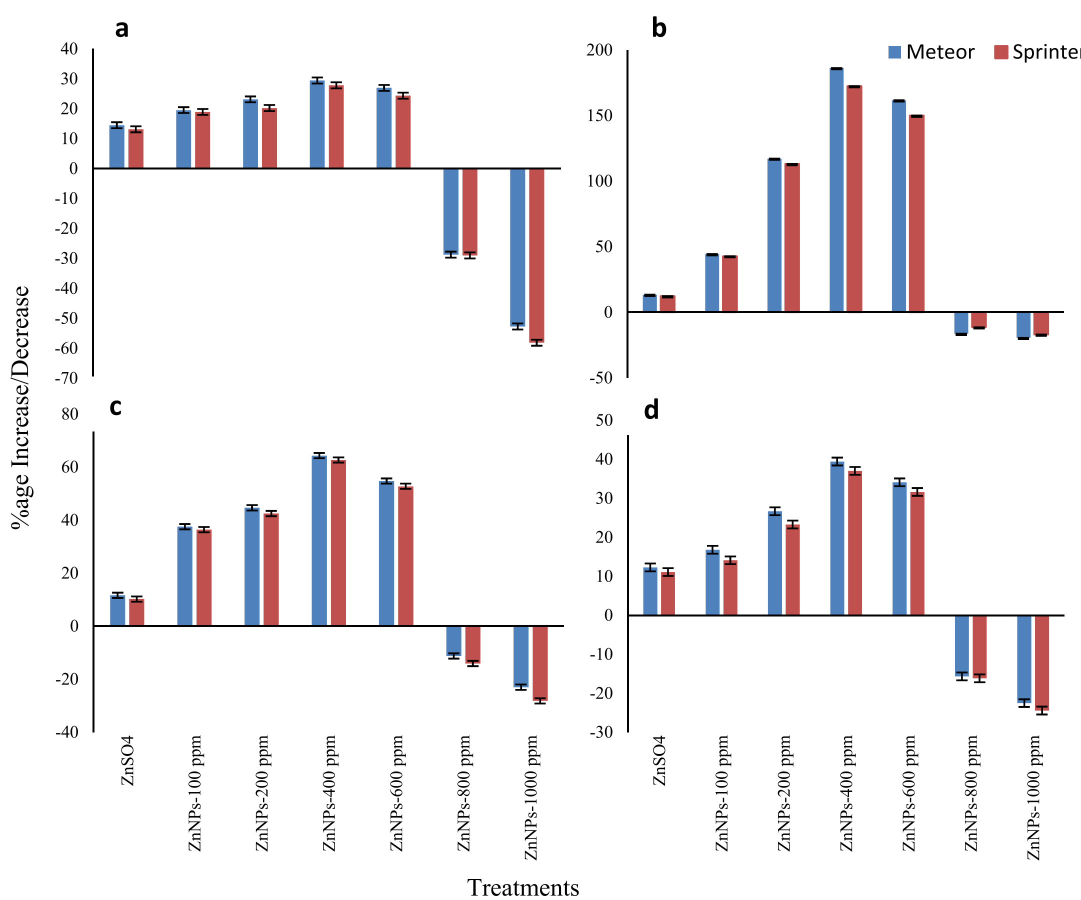

Fig. 2. Effect of different concentrations of Zinc Nanoparticles on a) shoot fresh weight b) root fresh weight c) fresh weight of leaves and d) total fresh weight of *Pisum sativum* L. (var. Meteor and Sprinter) harvested at 60 DAS given assoil drench.

3.3.2. Root, shoot and leaves dry weight

The dry matter production of pea obtained with different treatments of ZnNPs along with zinc sulphate application was significant at 400 ppm concentration for both varieties. The data indicated that there is a significant difference between control, zinc sulphate and different concentration of Zinc nanoparticles (in the case of root, shoot and leaves). Root, shoot and leaves dry matter production was adversely affected by Zinc nanoparticles concentration at 1000 ppm for both varieties of pea (Fig. 3).

The root, shoot and leaves dry weight of plants which were exposed to treatments showed the increasing pattern of increasing concentration of zinc nanoparticles. The shoot dry weight of plants treated with a soil drench of ZnNPs-400 ppm concentration showed a higher value (2.89 g; var. Meteor) than the dry weight of plants that were treated with ZnNPs-1000 ppm (0.78 g). The plant's given zinc sulphate (1.79 g) had less shoot dry weight as compared to zinc nanoparticles treatments but more than the control (1.66 g).

The leaves dry weight exhibited the same increasing trend that dry matter of root and shoot had shown. The highest at 400 and 600 ppm ZnNPs level, but it showed a gradual decline from 800 ppm level onwards as compared to zinc sulphate and control. The observed data of total dry weight for var. Meteor was in following manner 5.15 g > 4.9 g > 4.73 g > 4.27 g > 3.75 g > 3.23 g > 2.53 g > 1.65 g for 400 ppm, 600 ppm, 200 ppm, 100 pp, ZnSO4, control, 800 ppm and 1000 ppm. Similarly, the recorded data of total dry weight at 70 DAS for var. Sprinter was also in the same trend.

3.4. Productivity assessment

At 60 DAS the productivity of pea was estimated by checking the effect of zinc nanoparticles on it. The productivity had a direct relation to different concentrations of zinc nanoparticles. As the concentration increased the yield of pea was also increased but after certain concentration, the yield started to reduce as the concentration increased.

3.4.1. Number of flowers

The number of flowers was observed by giving the treatments to pea plants. Eight different treatments were given to two varieties of pea which were control, $ZnSO_4$, ZnNPs-100, ZnNPs-200, ZnNPs-400, ZnNPs-600, ZnNPs-800 and ZnNPs-1000 ppm. Among all these treatments the greater number of flowers were observed at ZnNPs-400 ppm while fewer numbers were recorded at ZnNPs-1000 ppm in both varieties.

In var. Meteor at ZnNPs-400 ppm the number of flowers were 8.67 which had more number than the application of zinc sulphate (3.33) and control (2.67). While in var. Sprinter the 7.67 was the maximum number of flowers which were observed at 400 ppm but 0.67 was the minimum number of flowers which were obtained at 1000 ppm. Overall, more number of flowers were produced by treated plants of var. Meteor as compared to var. Sprinter. Table 2 show the number of flowers in both varieties.

3.4.2. Number of pods and seeds Plant^{-1}

Eight treatments were used to check the effect of zinc nanoparticles on pea plants along with the application of zinc sulphate.

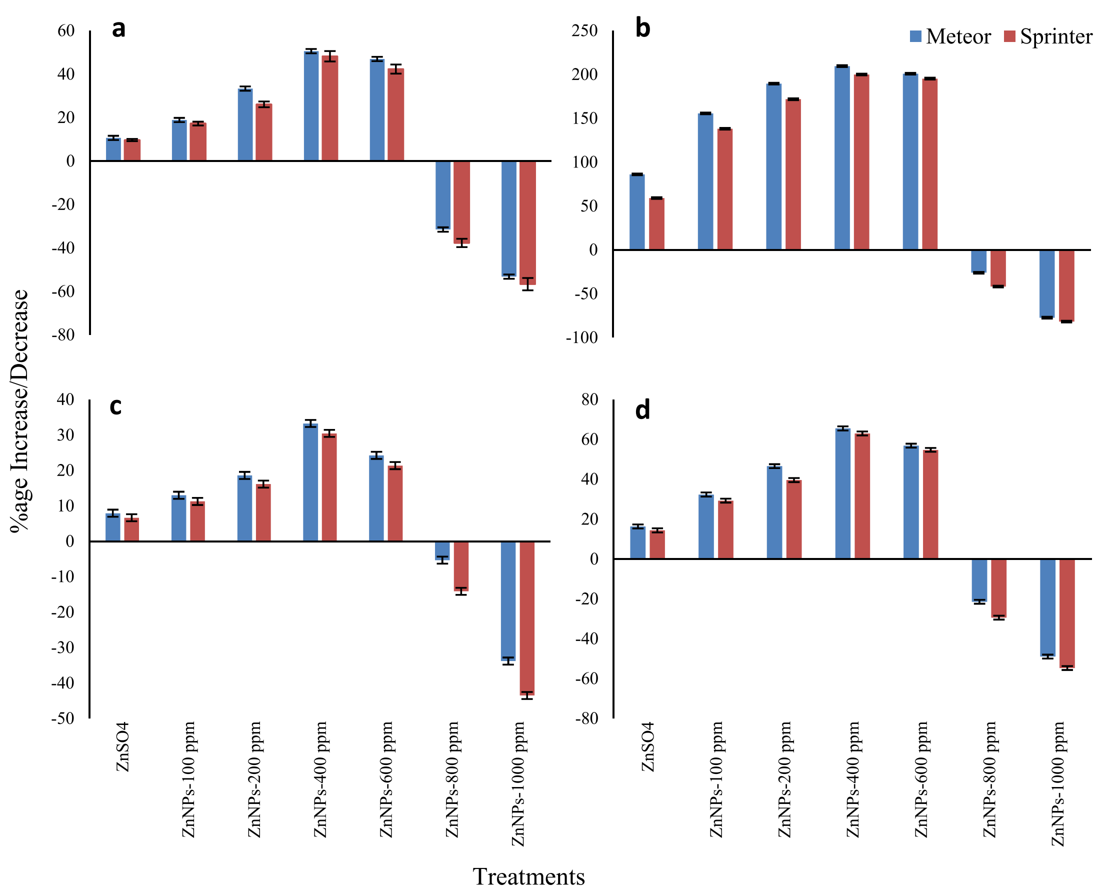

Fig. 3. Effect of different concentrations of Zinc Nanoparticles on a) shoot dry weight, b) root dry weight, c) dry of leaves and d) total dry weight of *Pisum sativum* L. (var. Meteor and Sprinter) harvested at 60 DAS given assoil drench.

Table 2
Productivity Assessment of *Pisum sativum* L. (var. Meteor and Sprinter) harvested at 60 DAS by using the soil drench under different zinc nanoparticles concentration during the growth season 2019.

Varieties of Pea	Treatments	Yield Parameters				
		No. of Flower	No. of Pods Plant^{-1}	No. of Seeds Plant^{-1}	No. ofSeedsPod^{-1}	Length of Pod (cm)
Meteor	Control	2.67d ± 0.3	1.33e ± 0.5	10.33e ± 0.2	7.33e ± 0.3	5.43f ± 0.1
	$ZnSO_4$	3.33d ± 0.2	1.67e ± 0.3	12.33d ± 0.3	7.67de ± 0.8	6.13e ± 0.3
	ZnNPs-100	4.67c ± 0.3	2.67d ± 0.1	15.67c ± 0.1	8.33bcd ± 0.6	6.53d ± 0.7
	ZnNPs-200	5.33c ± 0.4	3.67c ± 0.3	15.33c ± 0.7	8.67bc ± 0.9	6.97c ± 0.9
	ZnNPs-400	8.67a ± 0.6	6.33a ± 0.2	26.33a ± 0.4	10.33a ± 0.5	8.03a ± 0.6
	ZnNPs-600	6.67b ± 0.2	5.33b ± 0.1	24.33b ± 0.3	9.33b ± 0.4	7.43b ± 0.3
	ZnNPs-800	1.67e ± 0.4	1.33e ± 0.9	5.67f ± 0.6	5.67f ± 0.3	4.87 g ± 0.5
	ZnNPs-1000	1.33e ± 0.1	0.67e ± 0.8	3.67 g ± 0.5	3.67 g ± 0.2	4.37 h ± 0.3
Sprinter	Control	2.33e ± 0.2	1.33de ± 0.3	6.33f ± 0.3	6.67e ± 0.7	5.17f ± 0.2
	$ZnSO_4$	2.67e ± 0.8	1.67de ± 0.4	7.33e ± 0.1	7.33 cd ± 0.1	5.87e ± 0.1
	ZnNPs-100	3.67d ± 0.3	2.33d ± 0.6	12.33d ± 0.2	7.67bcd ± 0.3	6.23d ± 0.8
	ZnNPs-200	4.67c ± 0.2	3.33c ± 0.1	13.67c ± 0.6	8.33bc ± 0.5	6.83c ± 0.4
	ZnNPs-400	7.67a ± 0.1	5.67a ± 0.5	22.33a ± 0.8	9.67a ± 0.2	7.51a ± 0.3
	ZnNPs-600	5.67b ± 0.3	4.33b ± 0.3	18.67b ± 0.3	8.67b ± 0.1	7.23b ± 0.2
	ZnNPs-800	1.33f ± 0.5	0.67e ± 0.8	4.33f ± 0.1	4.33f ± 0.3	4.53 g ± 0.3
	ZnNPs-1000	0.67f ± 0.4	0.66e ± 0.6	2.67 g ± 0.3	2.67 g ± 0.7	3.87 h ± 0.1

Each treatment mean is sum of the three replicates and ± represents standard error (SE). Within each parameter values not followed by same letter are significantly different at Duncan's multiple range test and P = 0.05; ZnNPs; Zinc Nanoparticles.

Table 2 represents the number of pods and seeds plant^{-1}for both varieties. In both varieties the trend that was obtained in response to zinc nanoparticles are given below; ZnNPs-400 > ZnNPs-600 > ZnNPs-200 > ZnNPs-100 > ZnSO4 > control > ZnNPs-800 > ZnNPs-1000 ppm. So, the average values of the number of pods plant^{-1} for var. Meteor was 6.33 > 5.33 > 3.67 > 2.67 > 1.67 > 1.33 > 1.32 > 0.67. For number of seeds plant^{-1} were 26.33 > 24.33 > 15.33 > 15.67 > 12.33 > 12.33 > 10.33 > 5.67 > 3.67. For var. Sprinter the average values of number of pods plant^{-1} were 5.67 > 4.33 > 3.33 > 2.33 > 1.67 > 1.33 > 0.67 > 0.66 while the average values for number of seeds plant^{-1} were 22.33 > 18.67 > 13.67 > 12.33 > 7.33 > 6.33 > 4.33 > 2.67. As compared to var. Sprinter, more number of pods and seeds plant^{-1} were observed in var. Meteor.

3.4.3. Number of seeds Pod^{-1} and pod length

The average values of number of seeds pod^{-1} and pod length are given in Table 2 which shows that the maximum number of seeds pod^{-1} and pod length was obtained at ZnNPs-400 ppm while the minimum at ZnNPs-1000 ppm for both varieties. For var. Meteor the maximum number of seeds pod^{-1} and pod lengths were 12.33 and 8.03 cm while the minimum was 3.67 and 4.37 cm. The number of seeds pod^{-1} and pod length were increased with increasing concentration of zinc nanoparticles but after a specific concentration, the average values started to decrease. In the present research, the concentration of zinc nanoparticles started to become toxic after 600 ppm.

3.4.4. Pods, seeds and total fresh weight

The fresh weight increased with the increasing concentration of zinc nanoparticles (Fig. 4). Eight treatments were given including zinc sulphate and control. Maximum fresh weight was obtained at 400 ppm concentration while the minimum at 1000 ppm for both varieties. For var. Meteor the maximum percentage increase for the fresh weight of pods (38.37%) and seeds (69.34%) was recorded at 400 ppm while the minimum for pods (-59.77%) and seeds (52.55%) was recorded at 1000 ppm. The pattern that was recorded for percentage increase was given; ZnNPs-400 > ZnNPs-600 > ZnNP s-200 > ZnNPs-100 > ZnSO4 > ZnNPs-800 > ZnNPs-1000. The same trend was observed for the fresh weight of pods and seeds for var. Sprinter. The percentage increase of fresh weight of pods according to the above pattern was 20.78 > 13.87 > 7.11 > 3 > -44.52 > -60.93%. For seeds fresh weight were 60.70 > 24.40 > 12.73 > 12.92 > 2.87 > -40.28 > -53.25%. Total fresh weight also showed the same trend in response to different treatments.

3.4.5. Pods, seeds and total dry weight

Dry matter of pods and seeds were increased with increasing level of zinc nanoparticles (Fig. 4). The effect of zinc nanoparticles was more effective on pea varieties as compared to the application of zinc sulphate and control. Maximum dry weight was recorded at 400 ppm of zinc nanoparticles, but minimum was obtained at 1000 ppm. The maximum dry weight of pods (3.37 g) and seeds (1.74 g) were observed while minimum values were 0.49 g (pods) and 0.26 g (seeds) were recorded for var. Meteor. The percentage increase of dry matter of pods and seeds had a direct relation with different concentrations of zinc nanoparticles along with zinc sulphate and control for both varieties. The maximum percentage increase for pods and seeds were 260.54% and 123.38% at 400 ppm while the minimum was −57.71 and −123% at 1000 ppm. The same trend for total dry weight was recorded for both varieties.

4. Discussion

Environmental and nutrient problems related to chemical fertilizers are solved by nano fertilizers because conventional fertilizers are not able to fulfil the nutrient deficiency in plants as a result plants cannot grow properly. Particular nutrient deficiency in plants can be recovered by particular nanoparticles and this happens due to the presence of atoms on the surface of nanoparticles which exhibit different properties (Agarwal et al., 2017). Zinc nanoparticles play a significant role among other metals. These nanoparticles are not expensive to synthesise, easily prepared and environmentally safe (Hasan, 2015).

Results of the current study showed that a gradual increase in the level of zinc nanoparticles concentration significantly enhanced the plant growth and production in pea plant but after a specific concentration, it started to reduce the yield and plant growth. Among eight treatments, maximum growth was shown by plants at ZnNPs-400 ppm and 600 ppm as compared to bulk material while the minimum growth was observed at ZnNPs-800 ppm and 1000 ppm because at these concentrations zinc became toxic and suppress the plant growth. Subbaiah et al. (2016) observed that the leaf number of *Zea mays* L. increased as

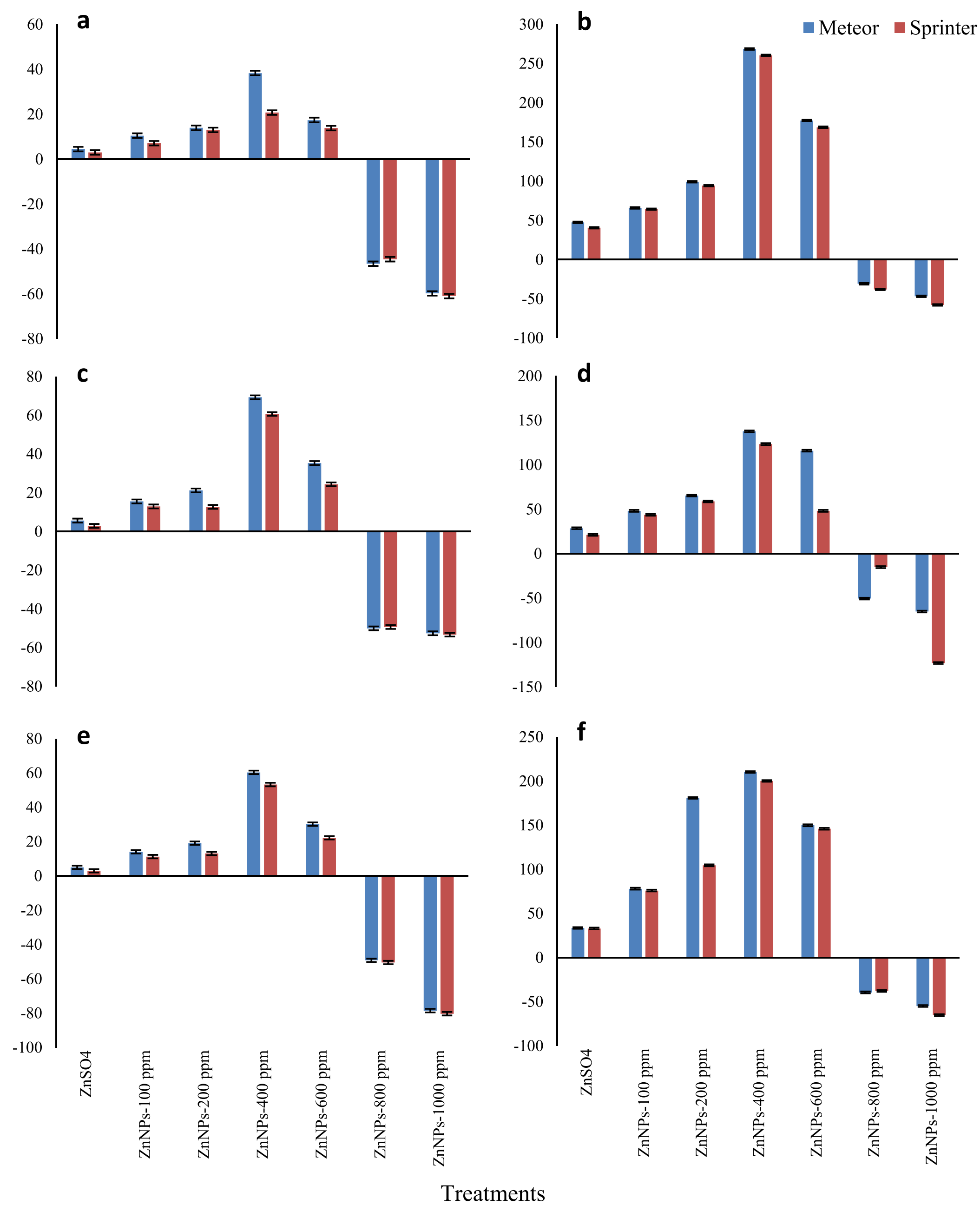

Fig. 4. Effect of different concentrations of Zinc Nanoparticles on a) fresh weight of pod plant^{-1}, b) dry weight pod plant^{-1}, c) fresh weight of seed plant^{-1}, d) dry weight of seed plant^{-1}, e) total fresh weight and f) total dry weight of *Pisum sativum* L. (var. Meteor and Sprinter) harvested at 60 DAS given assoil drench.

the level of zinc nanoparticles increased up to 400 ppm when treated with different concentrations of zinc nanoparticles (50, 100, 200, 400, 600, 800, 1000, 1500 and 2000 ppm along with control and bulk $ZnSO_4$). Dhoke et al. (2013) also reported that the area of *Vigna radiata* L. leaves increased as the concentration of zinc nanoparticles increased. Mahajan et al. (2011) reported that shoot and root length of mung bean (*Vigna radiate* L.) and chickpea (*Cicer arietinum* L.) increased with the increasing level of zinc nanoparticles concentration while there was a decrease in both shoot and root length as the concentration of zinc nanoparticles increased from a certain level. Nakasato et al. (2017) reported that pea growth parameters (number of tendrils and stipules) increased at the maximum level of zinc nanoparticles treatments as compared to control and zinc sulphate application and their number started to decline when the concentration increased from specific level. In current study same results were found out and accumulation of zinc nanoparticles started at high level and suppress the growth parameters of *Pisum sativum* L.

Narendhran et al. (2016) reported that the fresh and dry weight of root, shoot and leaves of *Sesamum indicum* L. increased gradually as the concentration of zinc nanoparticles increased. Pereira et al. (2014) and Oliveira et al. (2015) checked the effect of zinc nanoparticles on plant biomass *Brassica* sp. The phototoxicity of zinc nanoparticles decreased in fresh and dry weight of root, shoot and leaves at a medium concentration of nanoparticles.

Laware and Apparao (2010) checked the impact of zinc nanoparticles on number of flowers in okra. In 2014, Laware and Raskar checked the zinc nanoparticles effect on number of flowers in onions (Laware and Raskar, 2014). Number of flowers increased in response to different concentration of zinc nanoparticles and a high level of zinc cause toxicity. Number of pods plant^{-1} and number of seeds pod^{-1} were increased in response to three different concentrations of zinc nanoparticles in soybeans. With gradual increase in zinc nanoparticles concentration the number of pods and seeds increased (Jyothi and Hebsur, 2017). Another scientist, Prasad et al. (2012) reported that number of pods plant^{-1} and number of seeds pod^{-1} of peanut increased as a result of zinc nanoparticle concentration. Pods and seeds number increased with increasing concentration of zinc nanoparticles. The number seeds per cob of maize were significantly increased when zinc nanoparticles treatment was given. More number of seeds cob^{-1} were observed in zinc nanoparticles treated plants as compared to zinc sulphate treated plants in which less number of seeds cob^{-1} were observed (Taheri et al., 2015).

5. Conclusion

In the present century, the upcoming technology is nanotechnology which is operating in all fields of science. Now a day with the increasing population the demand for food is also increased, and the yield of staple crops is much low So, nano fertilizers are used to increase the yield of crops. So, it is the need of the hour to commercialize the metallic nanoparticles for sustainable agriculture. In the recent experiment, green synthesis of zinc nanoparticles by using *Syzygium aromaticum* L. for the estimation of their effect on growth and yield of *Pisum sativum* L. was done. Various growth and yield parameters of pea plants were increased as the ZnNPs treatments were increased. It is concluded that zinc nanoparticles can increase the growth and yield of pea plants as well as increase the productivity of staple crops. Growers are recommended to use ecofriendly and cost-effective green synthesized zinc nanoparticles for the achievement of better crop growth and production.

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