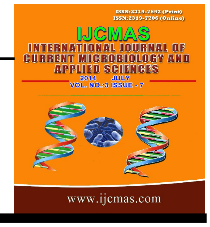

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Original Research Article

Influence of Zinc Oxide Nanoparticles on Growth, Flowering and Seed Productivity in Onion

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A B S T R A C T

Onion (*Allium cepa* L.) belongs to family Liliaceae and is one of the most important monocotyledonous, cool season vegetable crops in India. Seed production in onion requires 1.5-2 years so the unavailability of quality onion seeds is greatly responsible for its lower yield. The seed size and seed weight affect the final yield in onion. Furthermore, high quality seed is the critical input on which all other inputs depend for their potential yield. Six month rested onion bulbs (half cut) were planted in pots. The plants were sprayed 3 times at the interval of 15 days with graded concentrations of zinc oxide nanoparticles (ZnO NPs) along with sticker. The growth parameters like plant height and number of leaves per plant were determined at the time of flowering. The seed yield contributing parameters like number of seeded fruits per umbel, seed yield per umbel and 1000 seed weight was determined at the time of harvest. Seed samples obtained from NP treated plants along with control were tested for germination and early seedling growth. The plants treated with ZnO NPs at the concentration of 20 and 30µg ml^{-1} showed better growth and flowered 12-14 days earlier than the control. Treated plants showed significantly higher values for seeded fruit per umbel, seed weight per umbel and 1000 seed weight over control plants. This result indicates that ZnO NPs can reduce flowering period in onion by 12-14 days and even produce healthy seeds.

Keywords

Onion, ZnO NPs, Flowering, Seed yield.

Introduction

Onion (*Allium cepa* L.) belongs to the family Liliaceae and is one of the most important monocotyledonous and cool season vegetable crops in India. The unavailability of quality onion seed is greatly responsible for its lower yield. The seed quality parameters especially seed size and seed weight affect the final yield in onion production (Gamiely et al., 1990). Furthermore, high quality seed is considered as the critical input in onion on which all other inputs have to be managed for potential yield in onion.

Zinc (Zn) is an essential nutrient required by all living organisms and represents the

23rd most abundant element on earth (Broadley et al., 2007) and the 2nd most abundant transition metal, subsequent to iron (Jain et al., 2010). It is the required in six different classes of enzyme, which include- oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Auld, 2001). Zinc has been considered as an essential micronutrient for metabolic activities in plants and animals including humans. Although it is required in trace amounts in plants but, if it is not available in required amount, it creates physiological imbalances and affects enzyme activities and other metabolic processes (Baybordi, 2006). Zinc has important functions in the synthesis of auxin or indoleacetic acid (IAA) from tryptophan as well as in biochemical reactions required for formation of chlorophyll and carbohydrates. It also regulates the functions of stomata by retaining potassium content of protective cells. The crop yield and quality of produce can be affected by deficiency of Zn (Jamali et al., 2011). It was found that zinc has an important role in management of reactive oxygen species and protection of plant cells against oxidative stresses (Sheikh et al., 2009).

Researchers like Camp and Fudge (1945), Chapman (1966), Viets (1966), Anderson (1972), Mengel and Kirkby (1978), Marschner (1993), Brown et al. (1993) and Fageria et al. (2002) have demonstrated essentiality and role of zinc in plant growth, reproduction and yield. It has been indicated that the retention time of Zn in the plant system is low and hence, the bioavailability of Zn for long period is not sure with the use of $ZnSO_4$ fertilizer. Under high temperatures conditions $ZnSO_4$ has a large salt index and it may show burning injury if the plants are soft or sensitive. Farmers are using both zinc sulfate and EDTA-Zn chelate for soil and foliar applications; however, the efficacy is low.

Nanoparticles (NPs) with small size and large surface area are expected to be the ideal material for use as a Zn fertilizer in plants. Currently use of nanomaterials has been expanded in every fields of science including agriculture. It has been stated that application of micronutrient fertilizers in the form of NPs is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of fertilizer pollutions (Naderi and Abedi, 2012). It is because of that when materials are transformed to a nanoscale, they change their physical, chemical and biological characteristics as well as catalytic properties and evenmore increase the chemical and biological activities (Mazaherinia et al., 2010).

Reynolds, (2002) demonstrated that micronutrients in the form of NPs can be used in crop production to increase yield. Currently Prasad et al (2012) studied the effect of nanoscale zinc oxide on the germination, growth and yield of peanut and observed significantly more growth and yield. Therefore, in this study it was planned to study the effects of ZnO-TG NPs on the growth, flowering and seed production in onion.

Materials and Methods

Zinc oxide (ZnO) NPs about ≅18 nm sizes were synthesized by mixing 10 ml of sodium hydroxide (NaOH) solution (4mM) to 0.1 ml of 0.5 M solution of 1-thioglycerol and to 10 ml of 10^{-3} M solution of zinc acetate (Dhobale et al. 2008). The synthesized ZnO-TG NPs were dried in oven, suspended in water and then used for treatment in pot grown onion plants.

Six month rested onion bulbs (half cut) of same sizes were planted in pots. The plants were sprayed 3 times (15 days interval) with graded concentrations of nanoparticles along with sticker. The growth parameters like plant height, number of leaves per plant and days required for flowering were determined at the time of flowering. The seed yield contributing parameters like number of seeded fruits per umbel, seed yield (g) per umbel and 1000 seed weight (g) was determined at the time of harvest. The seed samples obtained from ZnO NP treated plants were tested for germination and early seedling growth (Raskar and Laware, 2013). Experiments were carried out in triplicate. Data recorded from three replications were subjected to single way analysis of variance (ANOVA) and critical differences were calculated at p=0.05 level.

Results and Discussion

Effect of different concentrations of ZnO nanoparticles on plant height, number of leaves per plant, and days required to flowering, seeded fruits per umbel and 1000 seed weight are given in table-1 and table-2. Results pertaining to plant height showed that maximum plant height was increased by 3.93 % i.e. 32.24 cm in 20 µg ml^{-1} concentrations of ZnO NPs over control and it was decreased by 0.45 % i.e. 30.88 cm in 40 µg ml^{-1} concentration of ZnO NPs as against 31.02 cm in control (Table no.1 and fig 1). Data on number of leaves per plant indicate that the number of leaves was significantly more in all the treatments and maximum number i.e. 23.14 was exhibited in plants treated with 30 µg ml^{-1} ZnO NPs as against 16.52 in control plants (Table-1). On the other hand it was observed that the number of days required for flowering of onion was significantly reduced in all the treatments of ZnO NPs. The plants treated at the concentration of 30 µg $ml^{-1}$of ZnO NPs initiated flowering after 51.44 days as against 66.28 days in control plants (Tale no. 1).

Significant differences in number of seeded fruit per umbel, seed weight per umbel and 1000-seed weight were observed in respect of NPs treatments (Table-2). This study reveals that the 30 µg ml^{-1} concentration of ZnO NPs produced the highest number (228.68) of seeded fruits per umbel whereas the least value (220.14) for the same parameter was recorded from 40 µg ml^{-1} concentration of NPs. The highest seed weight per umbel (2.34 g and 2.33g) was recorded from the 20 and 30 µg ml^{-1} concentration of NPs respectively, while the lowest seed weight (1.94 g) was obtained from the control plants. Again, the highest 1000-seed weight (3.52g) was obtained in 30 µg ml^{-1} concentrations of NPs and in control plants it was 3.18 g. The results of present investigation indicate that more vegetative growth of onion plants due to NPs treatments resulted in higher number of flowers and seeded fruits per umbel which might be the cause of higher seed weight per umbel and 1000 seed weight.

The results of seed germination test of seed lots obtained from ZnO NPs treated plants with respect to percent seed germination and seedling growth (i.e. radical and shoot lengths) along with control seed lot are given in table.3. The seed lots obtained from ZnO NPs treated onion plants showed significantly more seed germination i.e. 95.62%, 96.52% and 95.84% respectively in seed lots obtained from plant treated with 10, 20 and 30 µg ml^{-1} ZnO NPs as against 94.28% in control seed lot. Seedling performance with respect radicle and shoot growth in seed lots obtained from ZnO treated plants also showed same trend and highest increase i.e. 8.82% was noted in seed lot obtained from plants treated with 30 µg ml^{-1} ZnO NPs as compared to seed lot obtained from control plants.

Results depicted in table-1 and table-2 clearly indicate that ZnO NPs at 10 to 30 40 µg ml^{-1} significantly increased vegetative growth, induced early flowering and seed yield in treated onion plants. The increase in vegetative growth in onion might be due to fundamental role of Zn in protecting and maintaining structural stability of cell membranes (Welch et al., 1982) and use in protein synthesis, membrane function, cell elongation as well as tolerance to environmental stresses (Cakmak, 2000). Deore et al (2010) studied the effect of liquid organic fertilizer supplemented with chelated micronutrients (containing Zn) on red pepper and observed increased growth and yield. Similarly Datir et al (2010) studied the effect of organically chelated micronutrients (containing Zn) on growth and productivity in okra and reported increased growth and yield due to chelated micronutrient fertiliser. Datir et al (2012) also reported application of amino acid chelated micronutrients (containing Zn) for enhancing growth and productivity in chili (Capsicum annum L.)

Prasad et al (2012) suggested that ZnO NPs are absorbed by plants to a larger extent as compared to $ZnSO_4$ bulk. They also observed beneficial effects of NPs in enhancing plant growth, development and yield in peanut at lower doses, but at higher concentrations ZnO NPs were detrimental just as the bulk nutrients. Similar results were noted by Racuciu and Creanga (2007) on plant growth in *Zea mays* at early ontogenetic stages due to treatment of magnetic NPs coated with tetramethyl ammonium hydroxide.

It was noted that water repellence potential of leaf surface acts as one of the limiting factors, which can affect the Zn uptake through spray application processes (Holder, 2007). Higher solubility of metal ions in water might have limitation for their entry trough the lipophilic cuticle, but the permeability of lipophilic organic molecules trough cuticle increases with mobility and solubility of these compounds in the transport-limiting barricade of the cuticles. Hence, ZnO NPs with less hydrophilicity and more dispersible capacity in lypophilic substances can have more chances to penetrate through the leaf surface and release ions across the cuticle as compared to water soluble ions (Da Silva et al., 2006). The ZnO NPs used in present study were coated with TG; this indicates that they are less hydrophilic and more lypophilic in nature. Prasad et al., (2012) observed higher bioavailability of the ZnO NPs in peanut because of their nano size and lower water solubility. They reported that these properties of ZnO NPs are responsible to give higher yields in peanut compared to chelated $ZnSO_4$. Presence of NPs both in the extracellular spaces and within cells in *Cucurbita pepo* was also reported by Gonzalez-Melendi et al (2008). Abovementioned facts with respect to ZnO NPs and possibility of their penetration in onion leaf cuticle can elucidate their positive role on onion growth, flowering and seed yield.

In present investigation we have subjected the seed lots obtained from ZnO NP treated onion plants to germination test under laboratory conditions along with seed lot of control plants. The results pertaining to seed germination and early seedling growth of seed lots obtained from ZnO NPs are given in table-3. The seed lots obtained from ZnO NPs treated onion plants showed significantly more seed germination and seedling performance with respect radicle and shoot growth as compared to seed lot obtained from control plants.

Raskar and Laware (2014) studied effect of

ZnO NPs on seed germination and seedling growth in onion and observed that seed germination increased in lower concentrations of ZnO NPs but showed decrease in values at higher concentrations. Yilmaz et al. (1998) studied the effect of seed zinc content on grain yield and zinc concentration of wheat growth in zinc-deficient calcareous soils. They observed that wheat plants emerging from seeds with low Zn content had poor seedling vigor and field establishment on Zn-deficient soils. Rengel and Graham (1995) reported from pot culture experiments on wheat plants that increasing seed zinc content from 0.25 µg to 0.70 µg per seed significantly improved root and shoot growth under Zn deficiency.

Hence, it may be supposed that high Zn content in seed could act as a starter fertilizer and improved seed germination and early seedling growth. Ajouri et al (2004) noted that seed priming with Zn was very effective in improving seed germination and seedling development in barley. Deore and Laware (2011) studied effect of liquid organic fertilizer supplemented with organically chelated micronutrients (Zn, Cu and Fe) in red pepper and tomato and observed beneficial effects on seed germination and early seedling growth, they attributed these favorable effects to the availability of micronutrients to seeds during seed germination. Zinc enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen which is responsible for higher protein content. Zinc plays vital role in carbohydrate and proteins metabolism as well as it controls plant growth hormone i.e IAA. Zn is also an essential component of dehydrogenase, proteinase, and peptides enzymes as well as promotes starch formation, seed maturation and production. These facts indicate that the availability Zn to seed or high Zn content within the seeds during seed germination has very important physiological roles in seed germination and early seedling growth. The higher percent seed germination and significantly more seedling length in onion seedlings observed in seed lots obtained from ZnO NPs treated plants can be attributed to shipping of Zn from leaf tissues through phloem to the seed at the time of seed development and maturation process.

Table.1 Effect of ZnO NPs on growth and flowering in *Allium cepa*

Concentration of ZnO NPs (µgml^{1})	Plant height (cm)	PI/PDOC In height	Leaves Plant^{-1}	PIOC/PDOC In leaves	Days to flowering	PDOC in days for flowering
Control	31.02	0.00	16.52	0.00	66.28	0.00
10µg ml^{-1}	31.86	2.71	18.67	13.01	58.62	-11.56
20µg ml^{-1}	32.24	3.93	22.75	37.71	54.18	-18.26
30µg ml^{-1}	32.22	3.87	23.14	40.07	51.44	-22.39
40µg ml^{-1}	30.88	-0.45	19.76	19.61	56.26	-15.12
CD 5%	1.16		1.05		2.26	

CD= critical difference; PI/PDOC= percent increase/percent decrease over control

Fig 1: Effect of ZnO NPs on growth and Flowering - Plants treated with ZnO NPs at the concentration of 20 μgml^{-1} (Pot-3) and 30 μgml^{-1} (Pot-4) exhibited maximum growth and flowered 12-14 days earlier than the control (Pot-1)

Table.2 Effect of ZnO NPs on seed yield contributing parameters in *Allium cepa*

Concentration of ZnO NPs ($\mu g\ ml^{-1}$)	Seeded fruits Umbel^{-1}	PIOC	Seed weight umbel^{-1} (g)	PIOC	1000 seed Weight (g)	PIOC
Control	203.64	0.00	1.94	0.00	3.18	0.00
10$\mu g\ ml^{-1}$	212.04	4.12	2.05	5.67	3.22	1.26
20$\mu g\ ml^{-1}$	224.18	10.08	2.34	20.62	3.48	9.43
30$\mu g\ ml^{-1}$	228.68	12.29	2.33	20.10	3.52	10.69
40$\mu g\ ml^{-1}$	220.14	8.10	2.09	7.73	3.21	0.94
CD 5%	4.28		0.14		0.12	

CD= critical difference; PIOC= percent increase over control

Table.3 Seed germination test in seed obtained from ZnO NPs (μgml^{-1}) treated plants in onion.

Seed obtained from ZnO NPs (μgml^{-1}) treated plants	Percent seed Germination	PIOC	Shoot Length (cm)	Radicle length (cm)	Seedlings length (cm)	PIOC
Plants treated with 00 $\mu g\ ml^{-1}$ ZnO NPs	94.28	0.00	7.33	4.12	11.45	0.00
Plants treated with 10 $\mu g\ ml^{-1}$ ZnO NPs	95.62	1.42	7.46	4.38	11.84	3.41
Plants treated with 20 $\mu g\ ml^{-1}$ ZnO NPs	96.52	2.38	7.76	4.56	12.32	7.60
Plants treated with 30 $\mu g\ ml^{-1}$ ZnO NPs	95.84	1.65	7.78	4.68	12.46	8.82
Plants treated with 40 $\mu g\ ml^{-1}$ ZnO NPs	95.38	1.17	7.52	4.52	12.04	5.15
CD 5%	1.26		0.08	0.16	0.36	

CD= critical difference; PIOC= percent increase over control

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