

STUDY OF THE GROWTH RESPONSE OF SAFFLOWER CULTIVARS BY NANO-SILVER PRIMING

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Abstract

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Increasing seed germination and enhance seedling growth with expensing less time, cost and effort is vital for supply growing edible oil demand, in Iran. This experiment was carried out using a split plot layout in a randomized complete block design with three replications during 2013 growing season. The main factor was seed priming with various nano-silver particle concentration including 4 levels of 0 (distilled water: control), 20, 40 and 60 ppm, the sub factor was safflower cultivars with 3 levels included: Sina', Faraman' and IL111'. Results showed that the effects of nano-silver on growth traits including: stem dry mass, leaf dry mass, head dry mass, seed weight in head and numbers of head in plant were significant and the highest value of mentioned characters were achieved at 20 ppm nano-silver. The comparison of seed weight in head in various nano-silver particles treatments with control showed that the highest seed weight in head was obtained at 20 ppm nano-silver as it produced 63.18% more yield in comparison to control.

Key words: cultivar; growth; nanosilver particles; priming; safflower

Introduction

The estimated population growth from the current level of about 6 billion to 9 billion by 2050 (Hongda and Yada, 2011), results in growing food demand and increasing demand for edible oil. Therefore the technologies that conserve land and water by increasing yields with the same or fewer inputs and technologies that protect environmental quality are regarded. Priming is one of the methods used to increase seed vigor, improve germination and enhance seedling growth (Donaldson et al., 2001). So the farmers can reach a crop with better quality and more quantity with expensing less time, cost and effort (Vasilevski, 2003). Seed priming has important role in increasing the yield of different crops in relation to enhance in wheat, barley, upland rice, maize, sorghum, pearl millet, and chickpea respectively (Harris et al., 2005). The uptake efficiency and effects of various nanoparticles as seed priming on the growth and metabolic functions vary differently

among plants (Nair et al., 2010). The brand name 'nano-silver' is given to deionized water containing minute floating particles of silver ions in the size range of 10 to 100 nm (Nagender et al., 2008). It is proven that silver ions; inhibit the ethylene action by preventing its connection to its receptors in plant cells (Mishra et al., 2008). Silver eliminates unwanted microorganisms in farm soils and hydroponics systems. It has been used as foliar spray to prevent fungi, rot, moulds and several other plant diseases (An et al., 2008). Nano-Silver priming promoted growth of saffron in flooding stress by increasing root number, root length and leaves dry weight in study of Rezvani et al. (2011). Also improvement of growth and promoting photosynthesis and nitrogen metabolism of spinach by nano-TiO₂ (Titanium Dioxide) priming reported (Yang et al., 2006). Hediati and Salama (2012) showed that small concentrations of silver nanoparticles have led to an increase in shoot and root lengths, leaf surface area, chlo-

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rophyll, carbohydrate and protein contents in common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Using nano-silver had a significant effect on silver concentration in the plant shoot, the number of leaves, the height of the plant, plant dry weight, inflorescence dry weight, seed yield, and weight of one hundred seeds, polyphenol and tannin content in shoot of borage plant (Seif Sahandi et al., 2011). Among the different studies about nanoparticle priming, increasing of root and shoot biomass of mung (Dhoke and Mahajan, 2013), increasing nitrate reductase activity and acceleration of germination and growth of soybean (Lu et al., 2002), induced activity of specific antioxidant enzymes in *Brassica juncea* (L.) Vassiliū Matveievitch Czernajew seedlings (Priyadarshini et al., 2012), inhibition the biosynthesis of ethylene, and induced regeneration of multiple shoots from hypocotyl sections of cotton (Ouma et al., 2004), increasing growth of plant seedlings of mung and gram (Mahajan et al., 2011) was reported. Safflower (*Carthamus tinctorius* L.), an important oil-seed plant, is one of the oldest domesticated crops. India, Ethiopia, and Iran are the leading countries with the longest tradition of safflower cultivation as an oil crop (Tuncturk and Ciftci, 2004). Therefore due to huge need for more oil yield production in Iran, this research was carried out to study the potential of nano-silver seed priming in order to promote the growth and productivity of safflower cultivars.

Materials and Methods

This experiment was conducted using a split-plot based on a randomized complete block design with three replications at Research Field of Agriculture and Natural Resources Faculty, Islamic Azad University-Sanandaj Branch, during 2013 growing season, in the northwest of Iran (situated at 35°10' N and 46°59' E). The study area has a semi-arid climate with 450 mm annual precipitation in average. Soil type was clay with pH and EC equal to: 7.8 and 0.49 at the depth of 0-30 and 30-60 cm. In this study the main factor was seed priming with various nano-silver particle concentration: 0 (distilled water: control), 20, 40 and 60 ppm, the subfactor was safflower cultivars with 3 levels included as: 'Sina', 'Faraman' and 'IL111' that considered as subplot. The Silver nanoparticle (Nanocid L2000 colloidal liquid) was provided by Pars Nano Nasb Co, Ltd, Tehran, Iran. Seeds of safflower (*Carthamus tinctorius* L.) were obtained from the Sararod Rainfed Agriculture Research Centre, Kermanshah, Iran. The safflower seeds were soaked with different concentrations of nanosilver for 120-minute and they were spread in the shade until they were dried completely before planting (Tahmasbi et al., 2011). The field preparation was done

first by a 30 cm depth plough in April followed by disc and furrowing. The irrigation method was furrow systems. This experiment was conducted in 2 × 5 m plots, each of which having four cultivation rows with rows of 50 cm inter row and 5 cm intra row spacing and seeds were sown in the row in a depth of about 5 cm in 29 April 2013. In this study, at harvest stage, five plants from each plot were selected and some plant growth parameters such as: stem dry mass, leaf dry mass, head dry mass, head diameter, seed weight in head and numbers of head in plant were recorded. Finally data were statistically analyzed by SAS software package (SAS Institute., 2004) and mean comparisons were done according to Duncan's multiple range test method at the 0.05 probability level.

Results and Discussion

Stem dry mass

Stem dry mass was significantly affected by nanosilver priming (Table 1), as shown in Table 2 the biggest stem dry mass was obtained at 20 ppm nanosilver treatment. Also the lowest value was achieved by 60 ppm nanosilver. Nanosilver priming at 20 and 40 ppm increased stem dry mass as: 36.36 and 31.54%, over control, but with increasing nanosilver priming to 60 ppm it decreased to 34.49% less in comparison to control. These results are confirmed by findings of Ouma et al., (2004) that under the laboratory conditions, silver nitrate inhibited biosynthesis of ethylene, and caused regeneration of multiple shoots from hypocotyls sections of cotton. Dhoke and Mahajan, (2013) observed a 39.59% to 68.16% increase in root biomass and 44.09% to 83.92% increase in shoot biomass, over the control. In another study, Rezvani et al. (2011) found that soaking saffron corms with 40 or 80 ppm concentration of nanosilver, increased root length and leaves dry weight in flooding stress. Also, Yin et al., (2011) mentioned that increasing nanosilver concentrations caused a decrease in plant root growth, which indicated an increase in phytotoxicity of nanosilver particles. Furthermore Mahajan et al., (2011) studied the effect of nano-ZnO particles on the growth of plant seedlings of mung (*Vigna radiate* L. R. Wilczek) and gram (*Cicer arietinum* L.) and found that at certain optimum concentration, the seedlings displayed good growth over control and beyond that retardation in growth was observed. Hediati and Salama, (2012) studied silver nanoparticles on common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) and showed that small concentrations of silver nanoparticles had a stimulating effect on the growth of the plantlets, while the enhanced concentrations induced an inhibitory effect. However, increasing concentration of silver nanoparticles from 20 to 60 ppm has led to an increase

Table 1**Anova analyses for growth response of safflower cultivars by nano-silver priming, data are Mean of Squares (MS)**

SOV	Stem dry mass	Leaf dry mass	Head dry mass	Seed weight in head	Head diameter	Numbers of head in plant
Block	72.71 ns	0.42 ns	207.08 ns	7.7 ns	0.01 ns	52.54 ns
Nanosilver priming	1669.14 **	255.79 **	9195.9 **	1790.05 **	0.11 ***	13.24 ns
Ea	14.27	20.05	428.99	74.49	0.004	22.06
Cultivar (B)	570.12 **	29.49 ns	6780.25***	684.13 **	2.78 ***	60.12 *
Nanosilver priming × Cultivar	248.09 *	85.26 **	464.19 ns	521.48 ***	0.13 ***	155.67 ***
E	84.45	18.82	300.24	74.91	0.003	16.29
CV (%)	17.98	22.14	15.95	22.62	2.65	9.83

***, **, * Significant at 01%, 1% and 5% level an arrangement, ns: non-significant.

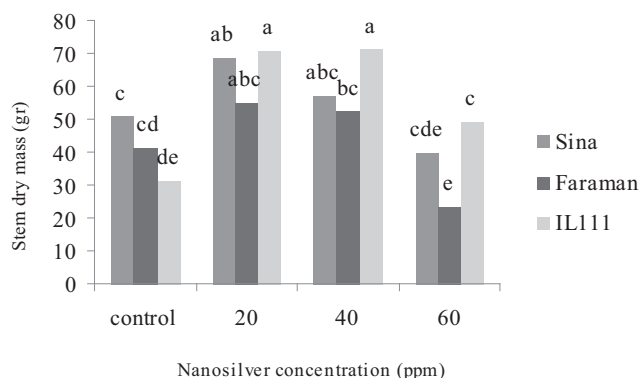
Table 2**The comparison of the means of growth response in safflower cultivars by nano-silver priming**

Treatment\ characters	Stem dry mass (gr)*	Leaf dry mass (gr)*	Head dry mass (gr)*	Seed weight in head (gr)*	Head diameter (cm)	Numbers of head in plant *
Nanosilver priming						
0% (control: distilled water)	41.37 c	15.56 b	85.27 b	32.7 b	2.45 a	41.58 a
20 ppm	65.01 a	27.45 a	138.32 a	53.36 a	2.35 b	42.32 a
40 ppm	60.43 b	17.79 b	133.95 a	45.61 a	2.28 c	39.48 a
60 ppm	37.64 c	17.56 b	77.05 b	21.42 c	2.19 d	40.82 a
Cultivar						
Sina	54.24 a	21.11 a	102.27 b	36.64 b	1.83 c	42.8 a
Faraman	43.21 b	17.98 a	88.71 b	31.67 b	2.33 b	38.53 b
IL111	55.9 a	19.68 a	134.95 a	46.5 a	2.79 a	41.82 ab

Mean with the same letters in column have not significant differences at 0.05 probability level by Duncan's multiple range test, *involved of 5 plant.

in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of the two tested crop plants. Additionally, the lowest values for these parameters were found with control plants, but the increased level of silver

nanoparticles resulted in the reduction of these compounds. In this study stem dry mass was affected significantly by cultivars as the highest stem dry weigh was found in 'IL111' and 'Sina' (Table 2). Also responses of cultivars were different among nanosilver concentrations. Finally the most positive effect of nanosilver in relation to increased stem dry mass was found in 20 and 40 ppm and IL111 cultivar (Figure 1).

**Fig. 1. Effect of nanosilver priming on stem dry mass in safflower cultivars.****Leaf dry mass**

Significant differences were found among nano-silver priming and leaf dry mass (Table1). The highest value for this character was found in 20 ppm nano-silver that increased leaf dry mass for 76.41%, over control (Tab 2). Also leaf dry mass was not different significantly among cultivars. Finally interaction of nano-silver and cultivars differ significantly, as the maximum leaf dry mass was obtained in 20 ppm nano-silver priming and Sina cultivar (Figure 2). Aso the same results were found by Hediati and Salama, 2012, Rezvani et al., 2011, Seif Sahandi et al., 2011, Zhen et al., 2005. Also,

An et al., 2008 reported the positive effect of nano-silver on extend maintenance period of leaves (from 2 to 21 days) in asparagus plant and they observed that, during this period the values of ascorbat, chlorophyll and fiber were higher in treated leaves.

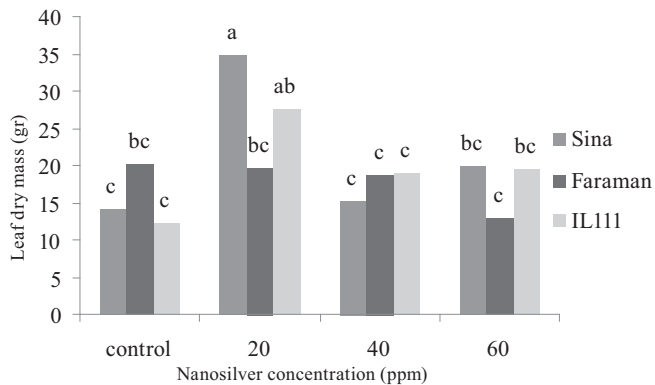


Fig. 2. Effect of nanosilver priming on leaf dry mass in safflower cultivars

Head dry mass

Head dry mass was affected by nano-silver; however seed weight was bigger in 20 ppm nano-silver (Table 1) and by increasing nano-silver concentration in 60 ppm treatment, seed weight decreased, even lower than control. Also this character was affected significantly by cultivars. This finding was in agreement with the results of other researchers. For example, Seif Sahandi et al. (2011) found that using silver (either as nano-silver or silver nitrate) had a significant effect on plant dry weight. Another study by Mahajan et al. (2011) reported the effect of nano-ZnO particles on the growth of plant seedlings of mung (*Vigna radiate*) and gram (*Cicer arietinum*). They found that at certain optimum concentration, the seedlings displayed good growth over control and beyond that retardation in growth was observed. The positive effect of nano-iron oxide on increasing pod dry weight of soybean was reported (Sheykhabagloo et al., 2010).

Seed weight in head

Seed and head weights were differ significantly (Table 1). The comparison of means in treatment with nano-silver various particles and control, showed that the highest seed weight at 20 ppm nano-silver (Table 2), it produced 63.18% bigger yield in comparison to control. Increasing nano-silver concentration as 60 ppm decreased the seed weight lowers than control. The finding of this study was in agreement with results of others. For example, Seif Sahandi et al. (2011) reports that seed abscission is one of the main factors causing reduced seed yield in borage plants.

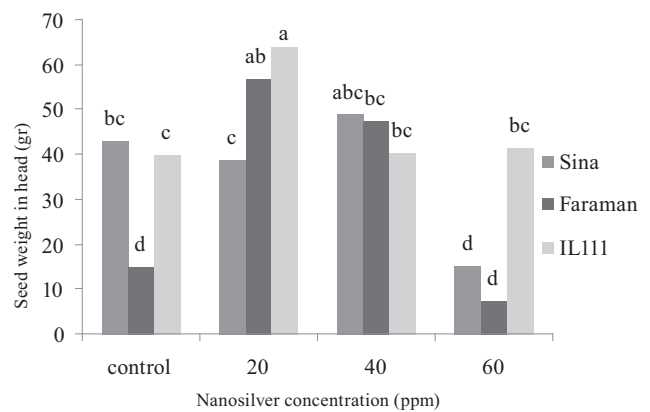


Fig. 3. Effect of nanosilver priming on seed mass in safflower cultivars

They observed increased seed weight as a result of reduced seed abscission due to the inhibitory effect of nano-silver at 20 to 60 ppm concentrations on ethylene action. Also, Purvis (1980) reported that ethylene caused an increase in enzyme activity of chlorophyllase and destruction of internal membrane of chloroplast, while that 100 ppm of silver nitrate caused a decrease in the production of ethylene and destruction of chlorophyll in calamondin fruit. Similar results were reported with the application of nano-iron oxide on soybean yield and quality, where concentration of 0.75 g/l, of nano-iron oxide increased, leaf + pod dry weight and pod dry weight. The highest grain yield was observed with using 0.5 g/l nano-iron oxide that showed 48% increase in grain yield in comparison with control (Sheykhabaglou et al., 2010). In this study the highest seed weight were recorded by IL111 cultivar, as it obtained seed weight increasing at the rates of 26.91 and 46.82% in Sina and Faraman cultivars, respectively. Finally, in this experiment, the highest seed weight was obtained in treatment with 20 ppm nano-silver and IL111 cultivar. The effect of nano-silver on seed weight of safflower cultivars is shown in Figure 3. These results were in agreement with Lu et al. (2002) who found that combination of nano-sized SiO₂ (Silicon Dioxide) and TiO₂ (Titanium Dioxide) could increase the nitrate reductase activity in soybean (*Glycine max*), increase its abilities for absorbing and utilizing water and fertilizer, promote its antioxidant system, and in fact accelerate its germination and growth. In another study Zheng et al. (2005) showed that the growth of spinach plants was greatly improved at 250–4000 ppm nano-TiO₂ concentrations, but there was no improvement at higher concentrations. They concluded that the significant effect of nano-sized TiO₂ is probably attributed to the small particle size, which allows its penetration

into the seed during the treatment period, helped the water absorption by the spinach seeds and as result of it, the germination of the seeds was accelerated and so exerting its enhancing functions during growth.

Head diameter

This character was affected significantly by nanosilver, cultivars and interaction of nanosilver and cultivars. Comparison of means showed that nanosilver had a negative effect on head diameter. The highest head diameter was achieved in control and was decreased with increasing in nanosilver concentration. According to the positive effects of nanosilver on increasing head and seed dry weight in this study, it seems that a negative relationship was between head diameter and head dry weight and numbers of seed in head (Table 2). Also the highest head diameter was induced in IL111 cultivars at all nanosilver treatments (Figure 4).

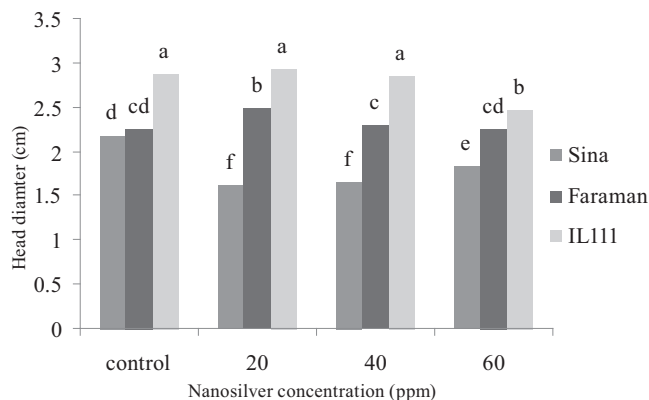


Fig. 4. Effect of nanosilver priming on head diameter in safflower cultivars

Numbers of heads in plant

Numbers of heads per plant did not differed significantly among nanosilver treatments (Tab 1). Increasing concentration of nano-silver from 40 to 60 ppm induced smaller number of heads in plant, even smaller in comparison to control. Other researchers got similar results, for example, Tahmasbi et al. (2011) showed that nano-silver treatment had the best effects at a concentration of 50 mg/L compared to the control. They concluded that nano-silver particle might have helped seed tubers to stay healthier for longer time in the soil and subsequently produced more vigorous plants. Also using silver had a significant effect on the number of leaves, plant dry weight, inflorescence dry weight, seed yield, polyphenol and tannin content in shoot of borage (Seif Sahandi et al., 2011). Another study by Mahajan et al. (2011) studied the effect of nano-ZnO particles on the growth of plant seedlings

of mung (*Vigna radiate*) and gram (*Cicer arietinum*). They found that at certain optimum concentration, the seedlings displayed better growth over control and beyond that retardation in growth was observed.

Also this character was significant among cultivars, as 'Sina' produced more heads per plant. Finally in this study, the biggest number of heads in plant was obtained at 20 ppm nano-silver treatment and IL111 cultivar, Figure 5. At all, silver can prevent ethylene action, and the study of Wilmo-wicz et al. (2008) showed that no flower bud formation was observed when seedlings were treated with ethylene, which was thought to be a strong inhibitor of flowering. Therefore, increased number of heads in nano-silver treated plants in this study may be due to the positive effect of silver in preventing undesirable ethylene action.

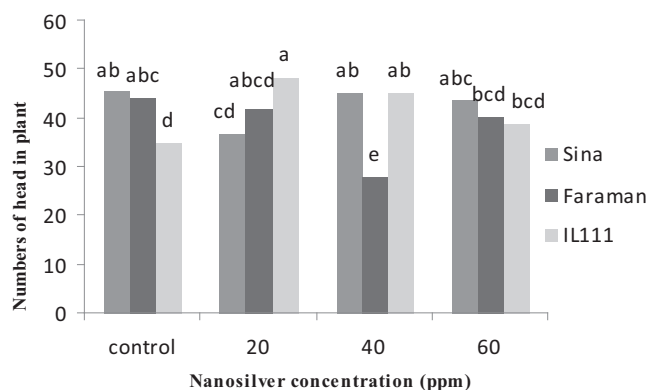


Fig. 5. Effect of nanosilver priming on numbers of heads in plant in safflower cultivars

Conclusion

The results of this study demonstrated the positive effect of nano-silver on some characters of safflower cultivars. Hence application of nano-silver at 20ppm concentrations had the biggest effect on seed weight. It produced 63.18% higher yield in comparison to the control. Also the highest seed weight was obtained by IL111 cultivar, as it produced 26.91 and 46.82% more yield as Sina and Faraman cultivars, respectively. In this study low concentration of nano-silver particles were increased by stem dry mass, leaf dry mass and head dry mass, and so seed weight increased. But at high concentration of nano-silver, we found the inhibitory and decreasing effects of nano-silver particles on the studied characters.

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