

Effect of Nano-Silver on Growth of Saffron in Flooding Stress

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Abstract—Saffron (*Crocus sativus*) is cultivated as spices, medicinal and aromatic plant species. At autumn season, heavy rainfall can cause flooding stress and inhibits growth of saffron. Thus this research was conducted to study the effect of silver ion (as an ethylene inhibitor) on growth of saffron under flooding conditions. The corms of saffron were soaked with one concentration of nano silver (0, 40, 80 or 120 ppm) and then planting under flooding stress or non flooding stress conditions. Results showed that number of roots, root length, root fresh and dry weight, leaves fresh and dry weight were reduced by 10 day flooding stress. Soaking saffron corms with 40 or 80 ppm concentration of nano silver rewarded the effect of flooding stress on the root number, by increasing it. Furthermore, 40 ppm of nano silver increased root length in stress. Nano silver 80 ppm in flooding stress, increased leaves dry weight.

Keywords—Flooding stress, Nano-silver, Saffron.

I. INTRODUCTION

SAFFRON (*Crocus sativus* L.) is a member of the family *Iridaceae*. A dry stigma of the saffron is the most expensive spice in the world [1]. Furthermore it has a widespread commercial use for food, aroma, coloring, cosmetic and potentially therapeutic purposes [2], [3]. Recent studies have shown that saffron is a good remedial and preventive agent for different cancers [4]. This plant is propagated only via the cultivation of corms since the flowers are sterile and unable to produce viable seeds [5]. It has 1–2 flowers with a lilac purple color [3].

At the beginning of saffron growth during autumn season, heavy rainfall can cause flooding stress, which is unsuitable for growth of most plants including saffron. This stress influenced many physiological, morphological and biochemical processes, including plant photosynthetic capacity, the amount of root and shoot growth, plant biomass production rates, water relations, carbohydrate metabolism, the cell structure, nutrition and balance between the hormones and affect gene expression [6, 7, 8]. Flooding stress causes oxygen deficiency (hypoxia) and accumulation of ethylene in plants which can inhibit root growth, root permeability and survival of most plants except wetland species such as rice [9], [10].

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Silver ions such as AgNO₃ have been recognized to inhibit ethylene action [11]. This effect of silver ions on ethylene was reported by several researchers [11], [12], [13], [14], [15], [16]. Silver eliminates unwanted micro organisms in farmer soils and hydroponics systems. It is being used as foliar spray to prevent fungi, rot, moulds and several other plant diseases. Moreover, silver is a great plant-growth stimulator, including silver salt, silicate and water soluble polymer to radioactive rays [17].

Nanotechnology with materials having unique properties that, has promised applications in various fields. It has provided new solutions to problems in plants and food science (post-harvest products) and offers novel approaches to the rational selection of raw materials, or the processing of such towards applying the disease control molecules, slow release of pesticides and developing diagnostic tools. Nano silver or Silver nano particles, is one of the most commonly used in the field of nanoparticles after carbon nano-tubes that every day is added in its application to the nano-world.

Silver nanoparticles mainly due to physical and chemical properties of that particular show in their use of electronic, optical, biochemical and pharmaceutical and health are frequently used. Moreover, the recent finding revealed that Ag nanoparticles can bind to the HIV [18] studied the effect of nano silver and silver nitrate on abscission and yield of seed in borage at seed growth stage. They reported that Raise in the concentration of nano silver from 20 ppm to 60 ppm has led to an improvement in the seed yield.

There is a little investigation about the effect of flooding stress on saffron. Keyhani and et al (2004) reported that, depending on the developmental stage, hypoxia/anoxia was a signal for an increase in either alcohol dehydrogenase or NAD-dependent lactate dehydrogenase in saffron [4]. However they did not report the effect of flooding stress on saffron growth traits.

Since new technology of silver nanoparticles allows economical application and practical use of this metal has created, this technology has been used in this study. Thus in this study, we tried to adverse the effects of flooding stress on growth of saffron by using silver nanoparticles. This study is the first research which is about nano silver effect on root induction of saffron corms in hypoxia condition.

TABLE I
ANALYSIS OF VARIANCE MEAN SQUARE TESTING TRAITS

S.O.V	D.F.	RN	RL	RDW	RFW	LBN	LBL	LBDW	LBFW
R	3	23.55 ^{ns}	0.97 ^{ns}	0.006 ^{ns}	0.051 ^{ns}	0.8 ^{ns}	0.9 [*]	0.001 ^{ns}	0.03 [*]
A	1	2090.91 ^{**}	39.56 ^{**}	0.027 ^{**}	2.55 ^{**}	1.72 ^{ns}	0.29 ^{ns}	0.008 ^{**}	0.001 ^{ns}
B	4	1549.41 ^{**}	7.21 ^{**}	0.002 ^{**}	0.45 ^{**}	2.73 [*]	3.11 ^{**}	0.004 ^{**}	0.05 ^{**}
AB	4	637.84 ^{**}	2.87 ^{**}	0.0002 ^{**}	0.058 [*]	0.93 [*]	3.58 ^{**}	0.007 ^{**}	0.06 ^{**}
E	27	46.92	0.63	0.0045	0.059	0.73	0.29	0.001	0.007
CV		14.66	9.4	14.2	14.6	13.2	12.8	10.9	11.4

* and ** respectively, significant levels of 5 and 1 %. A = Flooding stress and without flooding stress, B = Nano silver levels, AB = Interaction between A and B, RN = Root Number, RL = Root Length, RDW = Root Dry Weight, RFW = Root Fresh Weight, LBN = Leaf bud Number per corm, LBL = Leaf bud Length, LBDW = Leaf bud Dry Weight, LBFW = Leaf Bud Fresh Weight.

II. MATERIALS AND METHODS

This study was carried out in a factorial design with 4 replicates in the Greenhouse of Faculty of Agriculture Tarbiat Modarres University. Saffron corms were soaked with different concentrations of nano silver (water pretreatment, 40, 80 and 120 ppm) for 90-minute and then planting under flooding stress and non flooding stress conditions. The root number, root length, root fresh and dry weight, leaf bud number, leaf bud length, leaves fresh and dry weight were measured after 10 day. The data of this study was analyzed by SAS software and with a view the significant differences in variance Analysis (ANOVA), mean comparisons was done according to LSD test method.

III. RESULT

Flooding stress had a significant harmful effect on often traits including root number, root length, root dry and fresh weight and leaves fresh and dry weight (Table I). In contrast, the leaf bud number and leaf bud length have not affected by stress (Table I).

Root number: Under nonstress condition application of nano silver 40 and 80 ppm, increased the number of roots more than other treatments. Moreover, the effect of nano silver 120 ppm and water on this trait were similar together. Flooding stress reduced the root number. In these condition applications of nano silver 40 and 80 ppm over other treatments, were compensated the negative effects of flooding stress on the root number (Figs. 1 and 9).

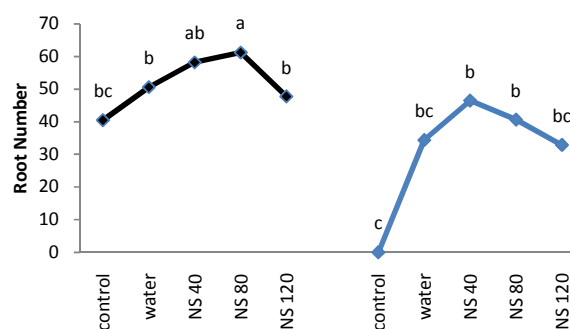


Fig. 1 Effect of various nano silver concentrations on root number under flooding stress and non stress.

Root length: in nonstress condition application of Water and nano silver 40, over other treatments were effective on root length, and they similarly increased it toward control in 99 percent confidence level. Flooding stress reduced the root length. Applications of Nano silver 40 ppm not only caused to compensate the adverse effects of flooding stress on the root length, but also increased it more than control in without flooding stress (Figs. 2 and 9).

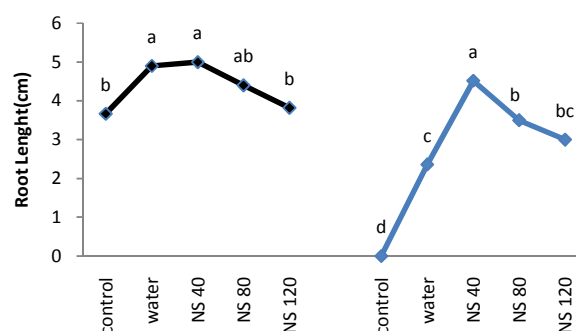


Fig. 2 Effect of various nano silver concentrations on root length under flooding stress and non stress.

Root dry weight and fresh weight: Under nonstress condition Water and nano silver 40 and 80 ppm, especially 80 ppm also increased to root dry weight and fresh weight were. After flooding stress, root dry weight and fresh weight were significantly less than those of unflooded controls. Nano silver 80 over other treatments increased this trait in stress situation (Figs. 3 and 4).

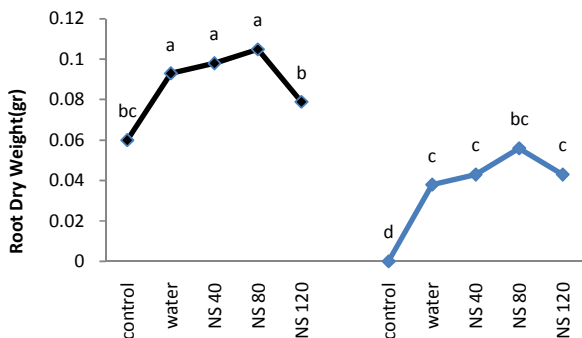


Fig. 3 Effect of various nano silver concentrations on root dry weight under flooding stress and non stress.

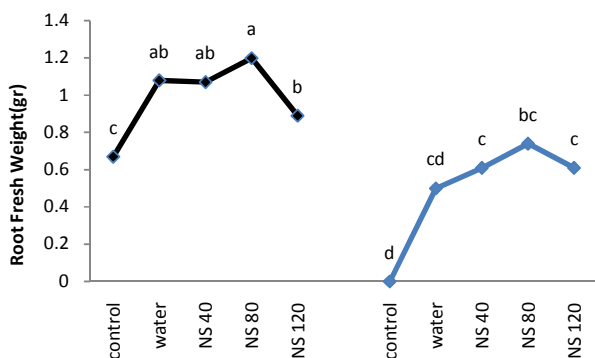


Fig. 4 Effect of various nano silver concentrations on root fresh weight under flooding stress and non stress.

Leaf bud number: Under nonstress condition Water pretreatment decreased leaf bud number but Nano silver 120 ppm enhanced it. Flooding stress had not any effect on the number of leaf buds per Corm. Nano silver 80 over other treatments increased this trait in stress condition (Fig. 5).

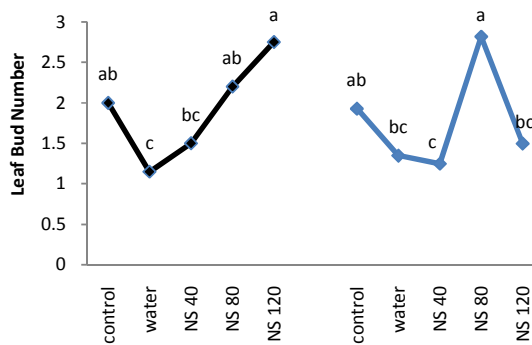


Fig. 5 Effect of various nano silver concentrations on leaf bud number under flooding stress and non stress.

Leaf bud length: Water pretreatment increased bud length in nonstress. Flooding stress had not any effect on it but nano silver 80 increased this trait in this condition (Fig. 6).

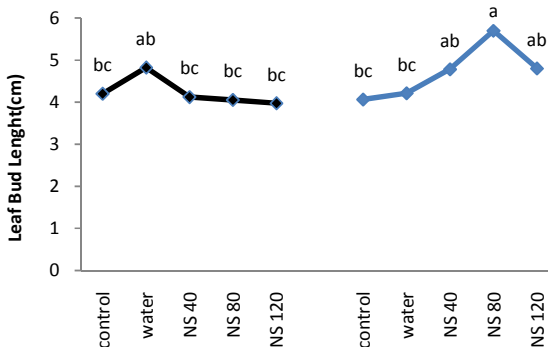


Fig. 6 Effect of various nano silver concentrations on leaf bud length under flooding stress and non stress.

Leaf bud dry weight: Nano silver 80 and 120 ppm increased bud dry weight But Water pretreatment decreased it in nonstress condition. Flooding stress reduced the leaf bud dry weight. Application of Nano silver 80 ppm compared to control in flooding stress, increased bud dry weight. Nano silver 120 contrary a positive effect on bud dry weight in non stress condition, reduced the amount of it in stress condition (Fig. 7).

Leaf bud fresh weight: Water pretreatment increased bud length in nonstress condition. Flooding stress reduced the leaf bud fresh weight. Nano silver 40, 80 and 120 ppm significantly enhanced fresh weight of buds in this stress (Fig. 8).

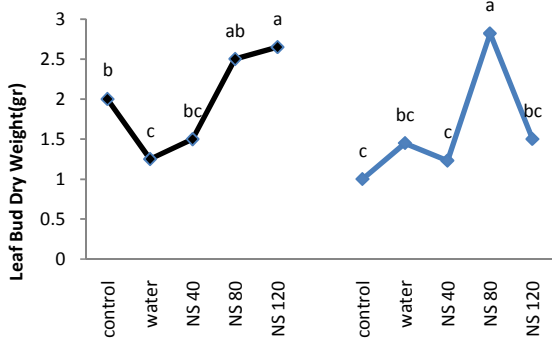


Fig. 7 Effect of various nano silver concentrations on leaf bud dry weight under flooding stress and nonstress

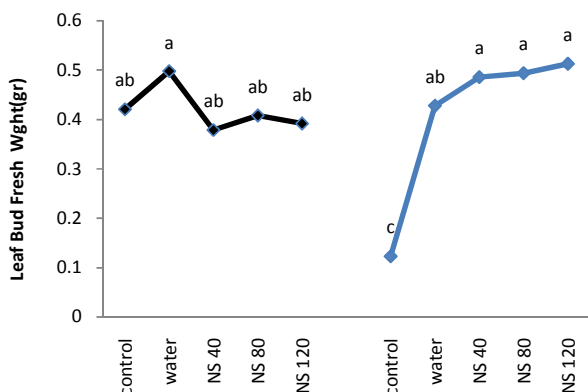


Fig. 8 Effect of various nano silver concentrations on leaf bud fresh weight under flooding stress and non stress

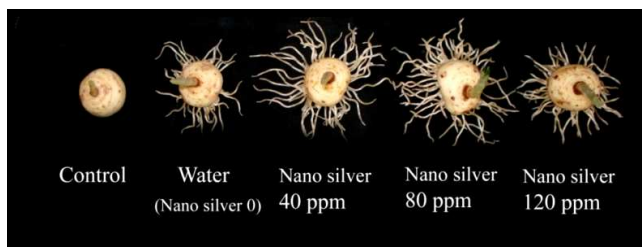


Fig. 9 Effect of different levels of nano silver concentrations on root growth characters such as root number and root length of saffron in flooding condition

IV. DISCUSSION

According to the results of these experiments, flooding stress has negative effects on the most traits measured in saffron plants, including the number of roots, root length, root fresh and dry weight, leaf bud fresh and dry weight. However, flooding stress did not affect the number of germinated leaf buds per corm and length of leaf bud (Table 1).

These results confirm our first hypothesis that flooding conditions decrease normal growth of *C. sativus*, while allowing it to survive, and our second hypothesis that flooding

stress has more adverse effects on root characters than leaf bud characters.

Deprivation of oxygen to the roots of plants is the main consequence of flooding. In most cases, oxygen shortage affects directly the roots and indirectly the shoots. Flooding stress changes soil chemistry. These changes include the lowering of soil redox potential (Eh) which translates into a progressively greater demand for oxygen within the soil and hence additional stress on the roots.

Flooding stress reduces the photosynthetic rate of numerous plant species [19], [20], [21], principally of flood-intolerant species. The decline in photosynthesis under flooding has been ascribed to stomatal closure, leaf chlorophyll decrease [19], ethylene production [22], and reduced sink demand [23]. Hongjun Chen and et al (2005) reported that Photosynthesis of flooded plants was about 60–70% of the rate of unflooded controls and Chlorophyll concentrations of flooded plants were about 60–70% of the unflooded plants during 15– 50 days of flooding in *Lepidium latifolium* plants. In addition this researcher understand that flooding stress reduced total biomass of *L. latifolium* and root/shoot ratio of flooded plants were significantly less than those of unflooded controls. Also Number of adventitious roots on the stem base increased with the duration of flooding [24].

Decreased rate of phloem transport to roots has been observed in flood- tressed crop species [25], which has been viewed as one cause of starch accumulation [26].

Adventitious root formation along the submerged stem portion, reduction in stem growth, wilting, chlorosis, epinasty, and abscission are the responses of flooded *Lycopersicon esculentum* [27]. Tang and Kozłowski (1982) also reported that after 30 days of flooding, leaf, stem and root biomass in flooded *Quercus macrocarpa* Michx. Seedlings were 95, 60 and 42% of the control plants, respectively, and the ratio of root to shoot in flooded seedlings was less than 50% of unflooded plants [28].

Unsuitable effects of flooding stress on saffron probably because of ethylene accumulation under flooding stress condition. The similarity between the reactions of plants to excessive watering and their response to treatment with ethylene is striking [29]. The connection between root hypoxia and the effect of ethylene was established when the roots of tomato plants where made anaerobic by the application of gaseous nitrogen, and an increased ethylene level was detected in plant shoots. The ethylene level increased only in those leaves that were vascular connected to the anaerobic roots [30], [31], [32] and [33]. Aquatic plants with slow root ethylene synthesis (e.g. rice) show a positive response to ethylene which decreases the level of abscisic acid, enhances tissue sensitivity to gibberellin and stimulates growth of submerged internodes [34]. Also Resistance of *Rumex palustris* plants to flooding is a consequence of both the rapid shoot elongation promoted by ethylene and the low sensitivity in this plant to ethylene [35]. In many other reports, researchers confirm the ethylene production in flooding stress [36], [37], [38].

Accumulation of ethylene in plants under waterlogging can inhibit root growth and survival of most plants except wetland species such as rice [9], [10], [39]. With various stresses in plants, the increase in ethylene synthesis serves as a common step in the chain of events leading to a variety of responses [36].

Ethylene biosynthesis is strongly regulated by internal signals and environmental stimuli from biotic and abiotic stresses, such as hypoxia [38]. Thus, by regulating the production or action of ethylene, the growth and development can be controlled in different unwanted stress such as flooding stress.

In these experiment applications of nano silver 40 and 80 ppm over other treatments, were compensated the negative effects of flooding stress on the most traits measured. Since the flooding stress increases ethylene production in most plants, therefore some results in this study can be referring to the effect of silver in preventing the ethylene action.

Many studies have indicated that silver ions, play a main role in influencing somatic embryogenesis, callus growth, shoot formation and efficient root formation and regeneration of both dicot and monocot plant tissue cultures [40], [41], [42], [43], [44], [45], [46], [47]. Beyer (1976b) reported that Silver ion is capable of blocking the action of exogenously applied ethylene in classical responses such as abscission, senescence and growth retardation [48]. This effect of silver ions on ethylene was reported by several researchers [11], [12], [13], [14], [15], [16]. Turhan (2004) studied the effect of AgNO₃ on potato plantlets. The results showed that MS basal medium supplemental with AgNO₃ resulted in an inhibitory effect on ethylene gas produced by potato plantlets [49].

Effects of AgNO₃ on in vitro root formation of *Decalepis hamiltonii* were studied. Addition of 40 µM AgNO₃ resulted in root elongation and initiation [44]. The effect of AgNO₃ on rooting and shooting was elucidated in *Vanilla planifolia*. Maximum number of shoots and highest shoot length was obtained on medium containing 20 µM AgNO₃. This matter not only induced shoot multiplication but also influenced rooting of vanilla explants [47]. Silver nitrate also induced rooting and flowering in vitro on *Rotula aquatic* Dipping of the basal end of shoots in NAA (2.69 µM) and silver nitrate (11.7 µM) solution improved rooting efficiency [50].

Inhibition of ethylene action by AgNO₃ stimulated regeneration of shoots from cotyledon explants of *Helianthus annuus* [51]. Ouma and et al. (2004) reported that under the in vitro conditions, silver nitrate inhibited biosynthesis of ethylene, and caused regeneration of multiple shoots from hypocotyl sections of cotton [52].

Ganesh and Sreenath (1996) reported in vitro sprouting of apical buds of *Coffea* under the influence of AgNO₃ [53]. The addition of N⁶-benzyladenine with AgNO₃ greatly enhanced the rate of sprouting. AgNO₃ enhanced in vitro shoot growth of *C. arabica* and *C. canephora* [47].

Silver ion mediated responses seem to be involved in ethylene, polyamines and calcium- mediated pathways, and play a important role in regulating morphogenesis including root induction. Use of silver ions can displace copper ions

from the receptor proteins. Moreover, it is confirmed that silver ions; inhibit the ethylene action by preventing its connection to its receptors in plant cells [54], [55], [56].

The reason of that we used nano silver, is the small size of nano particle, which causes more adhesion of nano particles to plant tissues. By considering the lesser use of silver in nano silver, this treatment can be used instead of other combinations of silver such as AgNO₃.

Note that the use of this material is more economically, use of this material with a concentration 40 ppm can reduce the risk of flooding stress in saffron plants.

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