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Review Article

Reviews of Salicylic Acid Mist Spray on Amount of Flavonoids, Anthocyanins, Sugar and Root Performance at Marigold Flower Species (*Calendula Officinalis* L) at Hydroponics System

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ABSTRACT

This research is performed in order of studying anti-stress effect of salicylic acid material on *Calendula Officinalis* L after germinating. Different Concentrations of salicylic acid (2,4,+ and 8 milimolar) on Seedlings of *Calendula Officinalis* L flower Hydroponic and greenhouse conditions during 4 days was studied. Between concentration of mist sprayed salicylic acid, 6 molar concentration had the best effect on Qualitative and quantitative performance of mentioned plant. Reviews indicate that high concntrations cause stop in growth of plant and Destruction of plant and also low concentrations had no particular effect. Results from measuring Flavonoids, anthocyanins and root performance showed that their rate after 24 hours in comparison to Control plants, indicates a meaningful increase. On treating salicylic acid there was a meaningful decrease on Germination of treated plants in comparison to control group was observed. Overally, salicylic acid at 6 molar concentration results in increase of Flavonoids, root, anthocyanins and Soluble sugars in comparison to control group.

Key words: epy Zhenin – anthocyanins - *Calendula Officinalis* L - salicylic acid

INTRODUCTION

calendula Officinalis .L is a yearling,fast growing plant belonging to the order of asterals , breed of asteraceae (composite) and sub-breed of asteroidea. From Asteraceae breed plants Producttions are gathered which have economic importance like Cooking oils, sunflower seeds, artichoke (Artichoke), sweetening materials and variety of teas. Is one of the most popular medicinal plants in the treatment of skin disorders, pain, and it is used as a bactericide, antiseptic and anti-inflammatory. This plant is widely grown to use its extraction at Herbal therapy. Extraction of *calendula Officinalis* .L has Pharmacological effects such as wound healing, anti-inflammatory, antibacterial, immunity stimulation, anti-tumor, anti-AIDS, etc. Anti-virus, anti-tumor, anti-mutagenic and antioxidant properties of marigold flowers have also been found. But now, one of the most uses of it, is in the treatment of inflammatory and skin diseases (Azzaz, et al., 2008). Marigold flowers with sepals, or without it, have been introduced as drugs in some Pharmacopoeia and they are used for Treatment of diseases of the stomach and the intestines, the coloring materials of the marigold, are in used in food industry to color Food products and some kinds of fats. In local medicine of Italy, *calendula Officinalis* .L is always used as anti-inflammatory and anti-fever. This plant is very effective in the treatment of conjunctivitis, pharyngitis, aphthous inflammation of the mouth (aphthous stomatitis), gingivitis and other inflammatory diseases of the skin and mucous membrane and also *calendula Officinalis* .L Contains flavonoids, which are antioxidants and protect the body from free radicals that destroy

cells. Main Combination of flowers include couple of fenol Compositions mainly Chlorogenic acid, flavonoids, ambely FEROON, EpyZhenin, quercetin, Batveltyn, luteolin and other flavonoids. The main terpenoids ingredients of it are α- biza bulol terpenoids and its oxides, and from azulens such as chamazulene. Salicylic acid or ortho-benzoic acid is from medium Shykmyk acid Cinnamic derivatives on synthesis of phenolic compounds. It is a phenolic compound which exists at nature and is also found on some plant tissues vastly. Salicylic acid is a natural phenol growth regulator of plant that regulates physiological processes. Most of the reviews performed about role of salicylic acid at plants is related to effect of this compound on improving or decreasing the effect caused by these stresses. Metabolites such as glutathione and ascorbic acid, decrease the caused effects of salicylic acid by effecting on Antioxidant enzymes such as catalase, superoxide dismutase, polyphenol oxidase, and peroxidase. About the role of Salicylic acid on sugars it can be said that during active photosynthesis and in the presence of light, the amount of carbohydrates that are produced as Triosephosphate in a leaf of plant, is more than the needed amount for energy production or synthesis as precursors. So the excess carbohydrates are converted to sucrose and transported to the other plant parts to be used at those parts as fuel. In most plants, starch is the original form of storage. But amino acids are also synthesized in plants and make polypeptides, in a study it has been observed that treatment with salicylic acid, inhibits the polysaccharide hydrolysis enzymes and Speeds up the formation of soluble polysaccharides. With this assumption salicylic acid increases the expense of insoluble to soluble sugars. However, soluble proteins level, i.e free amino acids such as proline are increased on aerial parts and roots of the plant treated with the salicylic acid. Salicylic acid regulate growth, division and cell death. In fact creates a balance between growth and aging (Horvath et al, 2002). Plants treated with salicylic acid increase cell division in root tip meristem and raise plant growth. In cell, Salicylic acid gets diactivated by two different methods: creation of link or metabolic diactivation in which large amounts of Salicylic acid is specified in shape of attached amide, ester, and glucoside types. But most of the attached form of salicylic acid is usually D-O-2-B glucocoPiranuzil Salicylic acid which is more abundant in stem than the rest of the organs. Glycosides lowest amounts of glycosides Salicylic acid is in the leaves. Plants can diactivate salicylic acid from metabolic aspect by hydroxylation of aromatic rings. Salicylic acid is produced by root cells and different micro-organisms and exists on various forms on surface of leaves and around root cells. Salicylic acid, is the inhibitor of catalase enzyme activity, which is a Cleansing hydrogen peroxide Enzyme and therefore with decreasing the activity of this enzyme results in increase of this material on planet. There are several defense mechanisms which have been emerged during Evolution. On these mechanisms is manufacture of specific compounds, such as flavonoids. In addition to their various roles in physiology, biochemistry and ecology of plants, flavonoids have a very important role in human nutrition. Flavonoids are produced from combination of shikimate and acetate route. Along the Shykymat route, L-aromatic amino acids of phenylalanine (L-Phe) is created. This amino acide during deamination by the enzyme of phenylalanine ammonialyase converts to building blocks of Phenyl propionic acids. Flavonoids are valuable natural compounds which biosynthesis on phenylpropanoid route. anthocyanins are Colored pigments, which get produced in result of a series of chemical reactions from flavonoids. It is also very unstable and easily susceptible to degradation. Anthocyanins stability is under the influence of pH, metal ions, Kopygmants, structure and anthocyanin concentration and the presence of other compounds such as flavonoids and other mineral materials. On this study the effect of different concentrations of salicylic acid on stimulation of biosynthesis of flavonoids especially epy Zhenin and anthocyanins in hydroponics environment of *calendula Officinalis* is studied.

MATERIALS AND METHODS

To perform present study marigold seeds were obtained from the Institute of Jihad Agriculture of Isfehan and they were cultivated in white pots

with dimensions of (height 20 cm and 25 cm diameter) in greenhouse of Research Center. Soil content of pots was selected by 1 to 1 ratio of sand and leaf soil, and they were examined at a maximum of 28 ° C and minimum of 15 ° C and initially they were irrigated for one month with manual fogging nozzle. To adapt and extraction of previously absorbed material, the for 10 days, they were transferred to hydroponic culture environment containing 1.2 Hoagland solution. Salicylic acid in different concentrations (2, 4, 6 and 8 mM) and distilled water as control unit, were given effect to the plant. The experiments were repeated for 3 times.

Measuring seed germination

In order of measuring flavonoids 0.1 grams of frozen cell mass in 3 ml of acid ethanol (ethanol and acetic acid in a ratio of 99 to 1) were well frayed and were centrifuged at g × 12000 for 15 min. Supernatant soluble was placed for 10 minutes in a hot water bath at 80 ° C. After cooling the sample absorption ratio was read by spectrophotometer at a wavelength of 270, 300 and 330 nm. To calculate the concentration of flavonoids, the extinction coefficient of 33000 cm-2 mol-6 was used.

Measuring total anthocyanins

To measure the anthocyanins, 2.0 g of shoot and root of frozen plant in 3 ml of acidic methanol (methanol and hydrochloric acid at a ratio of 99 to 1) were frayed and then the extract was centrifuged at g× 12000 for 15 min. Supernatant soluble was placed in darkness for one night and its absortion at wavelength of 550 nm was read with spectrophotometer devise. To measure the anthocyanins concentration extinction coefficient of 33000 cm-2 mol-6 was used.

Measuring soluble sugars

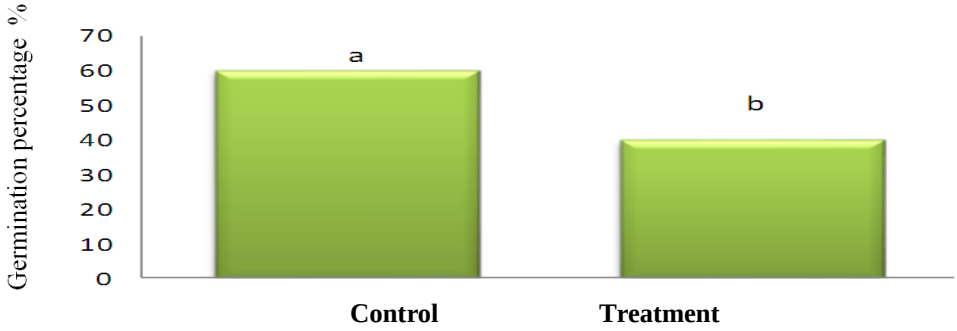
Frozen samples were extracted at 0.1 g on 3ml distilled water and then Homogeneous solution is filtered with application of filter paper. For the measurement sugar sample, 0.5 ml 5% phenol and 2.5 ml 98% sulfuric acid is added. Immediately after adding sulfuric acid, an exothermic reaction Along with the production of orange color happens that generates a lot of heat. Therefore, it is necessary after the addition of acid, to cool the reaction mixture for 10 minutes at room temperature. Standard curve by using different glucose concentrations from 0 to 20 micrograms per milliliter is drawn and absorption standards with total sugar absorption is measured using spectrophotometer devise on wavelength of 490 nm and amount of sample sugar, is determined based on Wet basis.

Statistical Analysis

This research was performed on a completely random design with 3 repeats. Analysis of Data was performed using MSTaTC and analisys variance of one-sided ANOVA was performed using dunken test within probability level of 1 %. Also drawing graphs was performed by using Excel software.

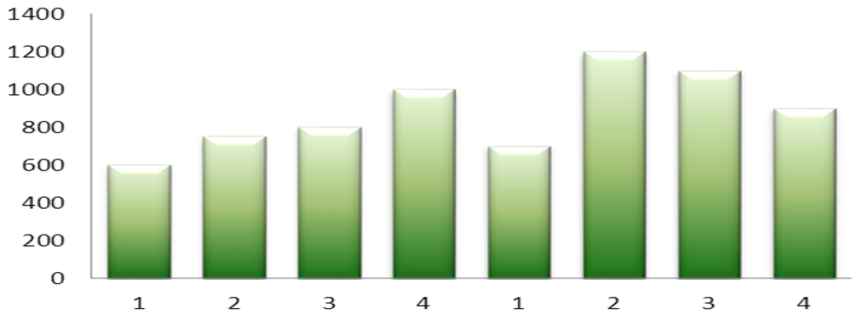
RESULTS:

Initially in order of finding appropriate concentration of salicylic acid for performing more studies, a wide range of concentrations (0,2,4,6,8 Molar) were given effect to the plant. Reviews showed that high concentrations results in inhibition of growing and destruction of plant. And also low concentrations did not have any particular effect on amount of flavonoids. Because of this very reason, 6 molar concentration was determined appropriate and was used in order of performing the tests. 3 repeats of this concentration were considered. 6 molar concentration of salicylic acid in comparison to control group caused inhibition of seeds germination. (Graph 1)

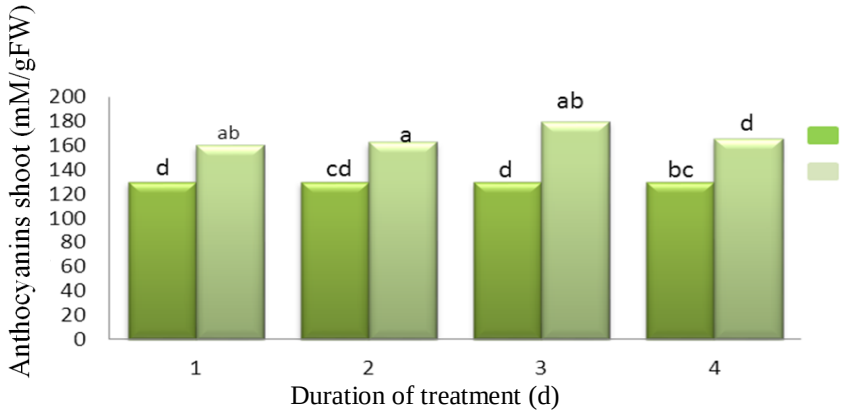


Graph 1: germination percentage of *Calendula Officinalis* L. average amounts of repeat are 6 repeats of Standard deviation. Different Letters indicate a meaningful Difference between averages at p<0.05.

Results from flavonoids measurements showed that on control samples there wasn't any meaningful Difference between total flavonoids during 4 days. While the second day in the samples treated with salicylic acid, flavonoids increased. It fell on the fourth day (see Figure 2).



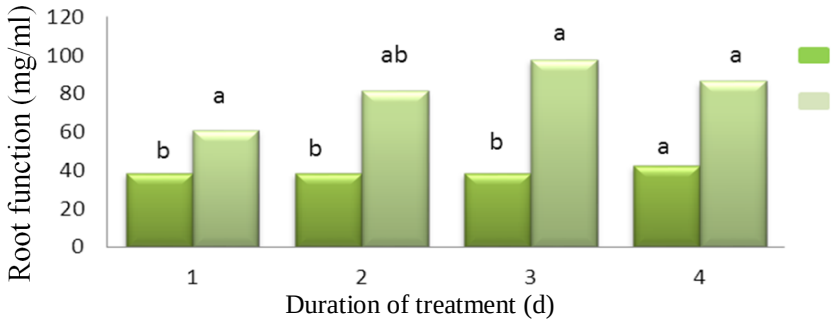
Graph 2: effect of salicylic acid on total flavonoids of *Calendula Officinalis* L Aerial organisms. Average amounts 3 repeats of Standard deviation. Same letters indicate there is no meaningful Difference between averages using dunken test on P<0.05 level.



Graph 3: effect of salicylic acid on Anthocyanin of aerial parts of *Calendula Officinalis* L. . Average amounts 3 repeats of Standard deviation. Same letters indicate there is no meaningful Difference between averages using dunken test on P<0.05 level.

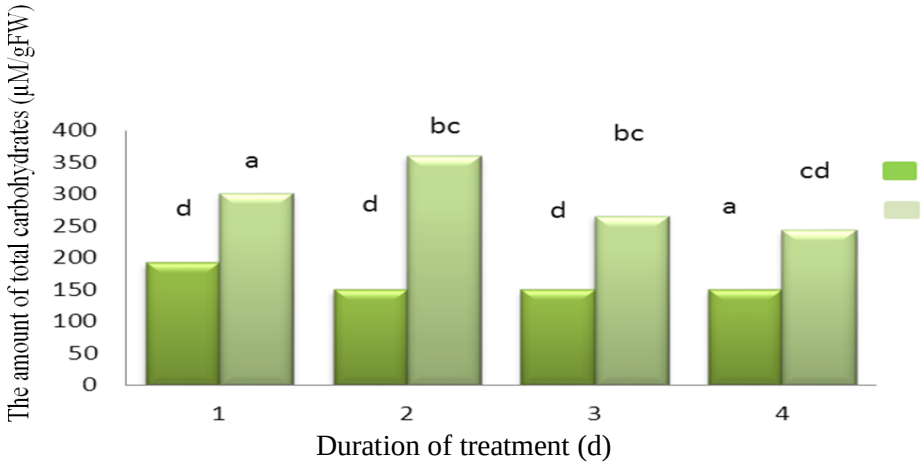
The results of the measurement of anthocyanins in shoots (Figure 2) showed that the amount of anthocyanin under influence by the concentration of 6 mM salicylic acid had an increasing process during the first 3 days and after that there is no meaningful Difference between its amount with control group (graph 3)

The results of HPLC showed that the concentration of 6 mM salicylic acid resulted in increase of egy zhenin on aerial organs in comparison to control group but no meaningful Difference was observed on epyzhenin amount during 4 days.



Graph 4: effect of salicylic acid on root performance of *Calendula Officinalis*. Average amounts 3 repeats of Standard deviation. Same letters indicate there is no meaningful Difference between averages using dunken test on P<0.05 level.

On plants treating of root performance had a significant increase in comparison to control plants. After 24 hours its amount reaches to the highest amount of itself which will continue until third day but after third day its amount almost reduces to the extent of control plants. (Graph 5)



DISCUSSION

Salicylic acid is a plant internal growth regulator which plays key roles in response to the environmental stress and making resistance in plants. The effect depends on the concentration of salicylic acid, the type of plant and its developmental stage. Despite that when it is used in higher concentrations, it is considered as a toxic factor for plant, which results in death of plant. When the pores are closed due to high concentration and thus they limit optical processes, available carbon dioxid os also reduced, so electron transformation in result of limited carbon dioxid reduces and thus power of assimilation is decreased.

Studies have shown that flavonoidsm, by reducing oxidative stress result in increase of plant tolerance to biotic and abiotic stress. Salicylic acid as stressor agent results in production of a whole range of flavonoids. Based on present study, external application of Salicylic acid after 24 hours to the concentration of 6 m Molar resulted in increase of flavonoids in plant which is in accordance by results of hayat and ahmad at 2005. It has been reported that Salicylic acid caused an increase of flavonoids in Aspirordela but in corn plant due to the robustness of this plant against 1 m Molar concentration, studying resulted metabolit secondary parameters did not have a considerable Difference.

Pastirova and Colleagues have observed the most amount of ambly feroon on 72 hours after application of 1 m Molar salicylic acid. On 50 and 250 Micro M concentrations there was no difference on combination of ambly feroon observed, but a very meaningful increase on amount of phenoc acides was observed.

Results of the comparing average showed that highest amount of root performance had been shown by consumption of 6 m Molar which had a 29.1% Superiority in comparison to control group.

On plants produce of anthocyanin is influenced by the interaction between internal and external factors such as light, temperature, carbohydrates, plant hormones and water. Therefore, we can consider the increase of production of anthocyanins on treatings of Salicylic acid consumption, because of increased auxin hormone and ultimately secondary produced metabolites and eventually these factors largely effect on amount of anthocyanin by effecting on Transcription factors. Studies of sudha and ravishankar showed Such that results of analysis of extracts prepared from the leaves of chamomile indicating that, salicylic acid treatment significantly increased the amount of anthocyanin on average comparison results which showed that treatment of 6 m Molar consumption resulted on 29.1 % Superiority in comparison to control group.

It is then understood that most amount of anthocyanins is related to 7 m Molar treatment of Salicylic acid. Salicylic acid had no effect on

anthocyanins amount in Non-green leaves of corn. On present study, according to graph 3 there is a meaningful difference between anthocyanins amount in control and treated samples. And these results confirm the effect of salicylic acid on production of anthocyanins. Salicylic acid is produced by root cells and various micro-organisms and exists in different forms on the leaf and around root cells. Root Constitutes 20% of the dry matter of the plant that Is responsible of absorbing water and nutrients from the depth of soil. According to obtained results on *Lolium temulentum* plant, treating plants with Salicylic acid increase Cell division in root tip meristem and increases plant growth. On this study also treatment with Salicylic acid with increase of concentration has resulted on production of more Assimilate materials and eventually higher performance of root on these treatments but in 8 m Molar concentration is caused reduction on Vegetative growth and thus reduction of manufactured assimilates for storing on root and decrease of root performance.

Overall amount of soluble sugars on treated plants compared to control plants had a meaningful increase which shows that different stresses can effect on secondary metabolits and in addition to initial metabolits by producing with Salicylic acid. There are reports which show that increase of growth and plants performance in result of anti-stress soulables application is because of their effect as an optical Breathing inhibitor on aerial parts. Also Salicylic acid with delaying on leaf senescence causes more photosynthetic activity in leaves and this results on increase of performance.

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