

# **The Effect of Silver Ions on Growth Changes, Chlorophyll and Total Phenolic Content in Soybean Plant (*Glycine Max L.*) Under Ethylene Treatment**

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**ABSTRACT:** Soybean is a native plant of eastern Asia and an important source of nutrition for cattle and human and its seeds contain protein, oil, carbohydrate and essential minerals. Ethylene is a gaseous hormone plays a role in some aspects of the plant life such as aging and leaf loss. The purpose of this study is to evaluate the effect of silver ions on the growth changes and some components of the soybean under ethylene treatment.

In this study, soybean seeds (type DPX) were planted in pots contained perlite with three random replications for each treatment. The treatment of AgNO<sub>3</sub> was in concentrations of 25, 50 μM for 10 days and the ethylene was in concentration of 100 and 300 ppm for 48 hours. The plants were taken out of the pots in 3-5 leaf phase and some morphological and biochemical parameters were measured. Studying the morphological, physiological and characteristic of soybean (*Glycine max L.*) showed that the ethylene treatment decreased the shoot fresh and dry weight and the chlorophyll amount while AgNO<sub>3</sub> treatment caused improving these parameters. AgNO<sub>3</sub> treatment increased total phenolic content significantly. Also ethylene treatment at 300ppm concentration led to the maximum decrease in growth and chlorophyll content while it had no significant effect on total phenol content. AgNO<sub>3</sub> at a concentration of 25, 50μM makes increase growth rate, the amount of chlorophyll in the presence of ethylene and also increasing the total phenolic content.

the production of secondary metabolites in plants can be increased by applying non- biological stimuli such as some metal ions. The positive effect of Ag ions on the growth and vitality of plant seems to result from the inhibition of ethylene activity.

**KEYWORDS:** AgNO<sub>3</sub>, Chlorophyll amount, Ethylene, Phenolic and flavonoid content, Soybean (*Glycine max L.*)

## **I. INTRODUCTION**

Soybean plant (*Glycine max L.*) is a member of the pea family that in the past half century, in terms of production after wheat and corn is in third place and in terms of nutritional value After corn in second place.

Soybean is an important source of nutrition for animals and humans and its seeds contain about 42% protein, 19/5% oil, 32% carbohydrates and minerals are essential[1]. Soybean potential as a functional food has caused an object to be used for research[2].

Secondary compounds are organic compounds that contrary to the initial compounds, their absence does not lead to instant death cell, but in the long time may lead to impaired survival and reproductive organism or its appearance will change. These compounds often play an important role in plant defense. Humans makes use of the secondary compounds as medicines and food flavorings. Terpenes, phenolic compounds, flavonoids, anthocyanins, tannins, isoflavones and compounds containing nitrogen (N) and sulfur (S), Are examples of secondary metabolites[3].

Production of secondary metabolites in plants can be increased with the use of non-biological stimuli like some metal ions[4]. Treatment with silver, because of the increased amount of flavonoids, to protect the plants against stress effectively[5].

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Ethylene is a gaseous hormone that plays a role in aging plants. This hormone is produced as a result of environmental stresses and their accumulation in plants reduced the growth and survival of most plants[6].

## II. RELATED WORK

### 2. Material and Methods

#### 2.1. Preparation and collect acorns for research

For this study, Agricultural Research Center Safiabad of Dezfool was produced seeds. Then for 20 minutes, seeds were soaked in distilled water in plastic containers containing perlite were planted at a depth of 5-3 cm. Pots were located in the greenhouse with aphotoperiod of 16 hours of light and 8 hours of darkness at a temperature of 27-25 ° C and relative humidity of 40%.

The seeds were watered with distilled water for about three weeks. Then irrigated with Hoagland solution was diluted ½, 3 times a week[7]. After three weeks, the plant samples were treated in the 5-3 leaf stage.

#### 2.2. Preparation treatments

In order to optimize the desired concentration, some plants were treated with different concentrations of 0,50,100 and 300 ppm ethylene for 48-24 hours. After 21 days, plants were treated with AgNO<sub>3</sub> treatment according to below chart:

Number of treatments	of	AgNO <sub>3</sub> (μM)	Ethylene (ppm)
Control		0	0
A		25	0
B		50	0
C		0	100
D		25	100
E		50	100
F		0	300
G		25	300
H		50	300

#### 2.3. Measurement of morphological parameters

Each plant per pot were removed and the roots were washed with distilled water. Shoots and roots of each plant were separated from each other to assess morphological parameters. In the vegetative stage, indices fresh and dry weight, root and shoot lengths and leaf area, were measured.

##### 2.3.1. Shoot fresh weight measurements

After removing the roots of the plant, shoot fresh weight was measured in grams.

##### 2.3.2. measuring dry weight

For measure this parameter, the aerial parts of the plant separately for 48 hours in an oven at 70 ° C were placed. After drying the samples, the dry weight was measured.

##### 2.3.3. Measure the length of root and shoot

Root and shoot lengths were measured separately with a ruler.

#### 2.4. Measurement of biochemical and chemical indicators

Biochemical assays were analyzed in this study include:

Amount of chlorophyll in leaves and measurement of total phenol content.

##### 2.4.1. Measurement of chlorophyll and carotenoid in the leaves

Measurement of chlorophyll and carotenoid was performed based on Lithenthaler1987[8]. With extraction of 0.1 g fresh leaf samples in 80% cold acetone and absorption at 664 and 647 nm (for chlorophyll a, b and total chlorophyll extract) and 470 nm for carotenoids was measured by a spectrophotometer . Pigments concentration in grams fresh weight and were tested using the following formula:

Chl a =  $12.25A_{664} - 2.79A_{647}$

Chl b =  $21.51A_{647} - 5.10A_{664}$

Carotenoids =  $(1000 A_{470} - 8.1 Ca - 85.02 Cb) / 198$

#### 2.4.5. The measurement of total phenolic content

The content of total phenolic compounds were estimated with using extraction method of Laima and Soland 1999[9] and absorption at 725 nm was measured for each sample and total phenolic compounds concentration in milligrams per gram of fresh weight.

#### 2.4.6. Total flavonoid content

To determine the amount of total flavonoid, aluminum chloride colorimetric method according to Zhishen method was used Zhishen et al., 1999[10]. The absorbance at 510nm was measured.

The flavonoids were calculated from the following equation:

$$Y = 0.0284x - 0.0024$$

#### 2.5. Statistical analysis

Data analysis was performed using SPSS version 18 and the average results were compared using Duncan test with 95%. Charts were drawn using a 2013 Excel software.

### III. EXPERIMENTAL RESULTS

#### 3.1. The evaluation of morphological parameters of soybean

The results of morphological parameters are shown in figure 3.1.

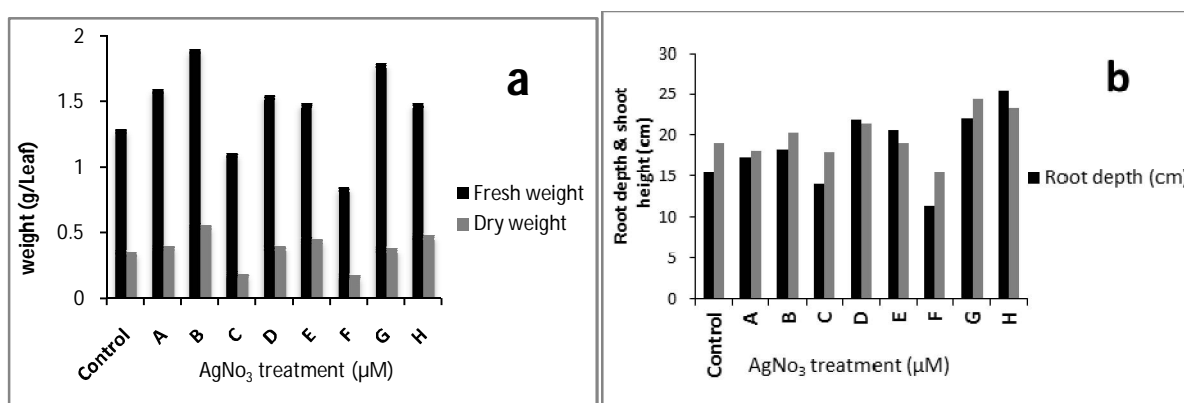


Figure 3.1. The effect of different concentrations of AgNO<sub>3</sub> and ethylene on morphological parameters weight (a), shoot height and root depth (b) in soybean

#### 3.2. The results of the measurement of biochemical and chemical indicators

##### 3.2.1. Photosynthetic pigments

The photosynthetic pigments content of soybean leaves is shown in figure 3.2. According to this chart, the treatment with 100 ppm ethylene decreased 25% and 300 ppm ethylene treatment, caused a 66% reduction in chlorophyll content of leaves than the control plants. The combination of AgNO<sub>3</sub> and ethylene treatments are significantly increased content of Cla than the plants that were only treated with ethylene. For this purpose, AgNO<sub>3</sub> treatment alone had no effect on Cla comparison with control plants.

The results of measuring the chlorophyll b content of leaves is shown in figure 3.2. Accordingly, the amount of Clb treated with 100 ppm ethylene is no change compared to control plants. Under treatment with 300 ppm ethylene at a rate of 71% decreased compared to control plants.

Treatment with 300 ppm ethylene plus 50 mM AgNO<sub>3</sub> is increases the amount of Clb than the plants that were only treated with the same concentration of ethylene. For this purpose, AgNO<sub>3</sub> treatment alone had no effect on the content

of Clb than the control plants. Also 25 and 50 mM AgNO<sub>3</sub> treatments combined with 100 ppm ethylene, affecting the content of Clb ratio to treatment plants with only 100 ppm ethylene. The results of measuring total chlorophyll content shown in Figure 3.2. Accordingly, 100 ppm ethylene treatment caused a 66% reduction in total chlorophyll content than the control plants and treated with 300 ppm ethylene was reduced 85% the total chlorophyll content than the control plants. Treated with 100 ppm ethylene, treatment with 25 mM AgNO<sub>3</sub> had been increase the total chlorophyll content of the plants that plant with only ethylene under the same treatment. The 25 mM AgNO<sub>3</sub> and 300 ppm ethylene treatment is increases the total chlorophyll content

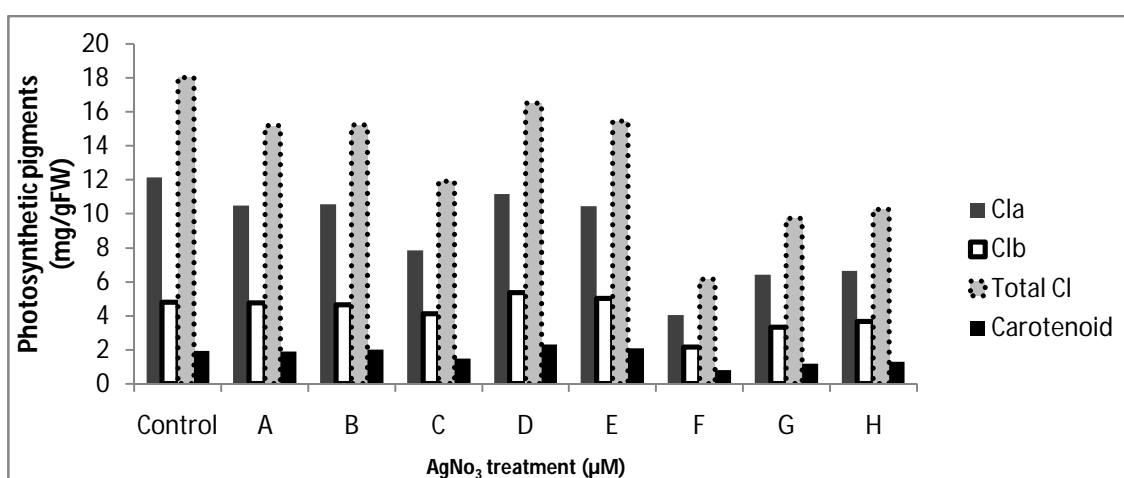


Figure 3.2. The effect of different concentrations of AgNO<sub>3</sub> and ethylene on Photosynthetic pigments (Chl a, Chl b, total Chl and Carotenoids) in soybean

### 3.2.4. The results of the measurement of flavonoids and total phenol content

The results of total phenol content is shown in Figure 3.3.b. Based on the results obtained, ethylene treatments significant impact on flavonoids and total phenol content than the control plants. But the treatments of 25 and 50mM of AgNO<sub>3</sub> was increased 125% total phenol content and also also increased the amount of flavonoids than the control plants. The treatment with silver nitrate and ethylene (both concentrations), has increased total phenol content than the control plants significantly but was not significant impact on flavonoids content

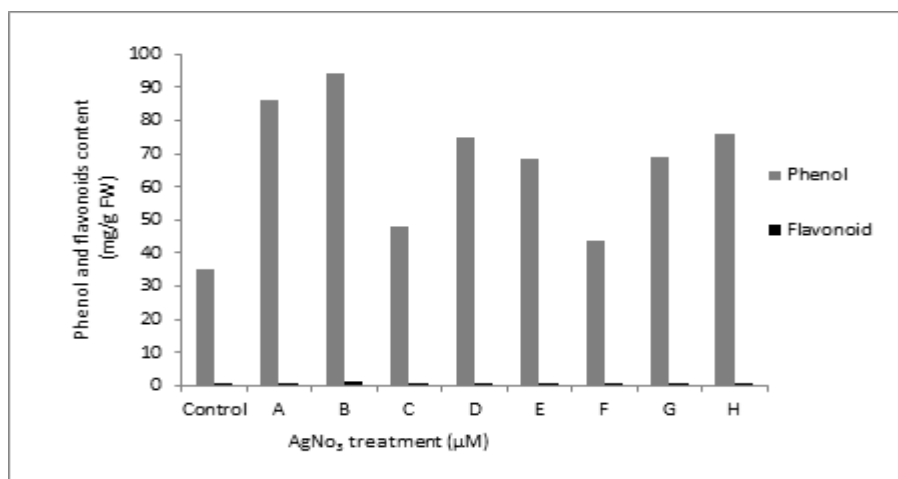


Figure 3.3. The effect of different concentrations of AgNO<sub>3</sub> and ethylene phenolflavonoids in soybean

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## IV. CONCLUSION

The investigation and the results of the parameters in this study were observed, treatment with 300 ppm ethylene with an impact on fresh and dry weight reduction, reduce the length of roots and shoots and reduced chlorophyll content causing tension in the treated plants were compared to control plants. While  $\text{AgNO}_3$  treatment, especially at a concentration of 50  $\mu\text{M}$ , enhances the growth parameters. So given that the concentration of 25  $\mu\text{M}$  of  $\text{AgNO}_3$  has a positive effect on plant conditions, but it seems that 50  $\mu\text{M}$  concentrations of  $\text{AgNO}_3$  is better effect in reducing the deleterious effects of ethylene on plants. In this study, treatment with 50  $\mu\text{M}$  of  $\text{AgNO}_3$  alone significantly increased fresh and dry weight, increasing the total phenol content and the amount of flavonoids. That it would be due to the effect of silver ions on the promoter is some specific genes.

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