

## **EFFECT OF SILVER NANOPARTICLES ON ZEA MAYS SEEDLINGS UNDER DROUGHT STRESS**

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## Abstract

Drought is a global dilemma caused by low average precipitation recorded in an area. Various artificial mechanisms are used to enhance plants resistance to drought conditions. Nanotechnology offers a wide range of modern techniques that improve and modify the already existing plant management technologies. For this work, silver nanoparticles (AgNPs) were prepared from silver nitrate in the presence of leaves Habaq mint which was utilized as reducing agent. The produced AgNPs were added to soil in which *Zea mays* were planted. The photosynthetic pigments, proline content, protein content, total carbohydrates and total antioxidant capacity have been monitored during the growth period of the shoot and root of the investigated plant. Also, Change of *Zea mays* leaves ultra-structure was investigated using transmission electron microscopy. The analysis of the parameters tested showed that additions of 10 ppm AgNPs are beneficial to the growth of *Zea mays* seedlings. However, increased concentrations of AgNPs to 150 ppm resulted in stunted to no growth. Based on these findings, it can be concluded that 10 ppm of AgNPs was able to stimulate the lengths and weights of the studied plant. Photosynthetic pigments decreased with increasing of AgNPs concentrations, whereas high shoot carbohydrate content was recorded in 150 ppm with 30% drought level. Proline and total antioxidant capacity increasing with 150 ppm, while decreasing at 10 ppm AgNPs concentration. We recommended that plants related to *Zea mays* species and a different preparations of AgNPs has to be tested.

**Key words:** Silver nanoparticle, drought stress, *Zea mays*.

### المستخلص:

يعتبر الجفاف مشكلة عالمية ناتجة عن انخفاض متوسط هطول الأمطار المسجل في منطقة ما، وهو يؤثر بشكل كبير على النباتات والحيوانات بسبب اعتمادها على إمدادات المياه. لكن النباتات لديها آليات طبيعية تساعد على التكيف في مثل هذه الظروف، ويمكن أيضاً استخدام الآليات الصناعية والهندسة الوراثية لجعل النباتات أكثر مقاومة للجفاف.

تقنية النانو - على سبيل المثال - هي طريقة تقنية حديثة تستخدم لضمان إنتاج ما يكفي من الغذاء من خلال مجموعة من التقنيات الحديثة التي تعمل على تحسين جودة الإنتاج، ومن الأمثلة الجيدة على ذلك الجسيمات النانوية الفضية. وقد تم تحضير الجسيمات النانوية الفضية باستخدام نترات الفضة وأوراق النعناع التي تم فيما بعد تطبيقها على بادرات الذرة وذلك بطريقة النقع والري. وتم تحليل أصباغ التمثيل الضوئي، وتعيين محتوى البرولين، والكربوهيدرات ومجموع نشاط المواد المؤكسدة على نمو بادرات نبات الذرة. أظهرت النتائج أن الجسيمات النانوية الفضية بتركيز قدره ١٠ جزء في المليون أثرت إيجابياً على إنبات بذور نبات الذرة، كما أظهرت أيضاً أن التركيزات العالية من جسيمات النانوية الفضية ١٥٠ جزء في المليون تسبب تقزم نمو بادرات نبات الذرة وقلة تحملها لظروف الجفاف.

كما أكدت النتائج أن تركيز ١٠ أجزاء في المليون من الجسيمات النانوية الفضية كانت قادرة على تحفيز وزيادة أطوال وأوزان نبات الذرة، مما يشير إلى أن الأسمدة النانوية يمكن أن تساعد النباتات في التغلب على ظروف الجفاف ومواصلة النمو، وعلى هذا الأساس يوصى باختبار النباتات ذات الصلة بنبات الذرة وتطبيق أنواع مختلفة للجسيمات النانوية الفضية.

**الكلمات الدالة:** الجسيمات النانوية الفضية، إجهاد الجفاف، نبات الذرة.

## 1. Introduction

Agricultural drought occurs when the soil moisture content is insufficient to satisfy the water requirements of a particular crop over a specific time span (Chowdhury *et al*, 2016). Saudi Arabia has been negatively impacted by drought. Intergovernmental Panel on Climate Change (IPCC) projects that the frequency and severity of drought are bound to increase because of the escalating worldwide climate change trends (Almazroui *et al*, 2012). The drought has many potential effects on plants including impeding their normal growth, interrupting water retention as well as reducing their ability to use water efficiently (Farooq *et al*, 2012). Nanotechnology refers to an essential modern research field that deals with designing, synthesizing, and manipulation of particle structures that range from about 1-100 nm. This form of technology comprises the general understanding of the various fundamental chemistry, physics, technology, and biology of various nanometer-scale particles (Singh *et al*, 2012). Taran *et al* (2017) note through nanotechnology, there is a new development of essential functional materials, new methods, and instruments used in increasing food security, more product development and several other new developments that relate to the industry. Silver nanoparticles (AgNPs) have unique physical, optical, biological as well as chemical properties that make them appropriate for food processing, healthcare products, electronics and industrial purposes (Iravani, 2011). In the present work, we present an investigation the impact of different concentrations of green synthesis silver nanoparticles 0 ppm, 10 ppm, 50 ppm and 150 ppm, on *Zea mays* (maize) seedlings under three drought levels 80%, 50% and 30% for fourteen days. After those certain morphological parameters along with some physiological parameters was determined.

## 2. Experimental

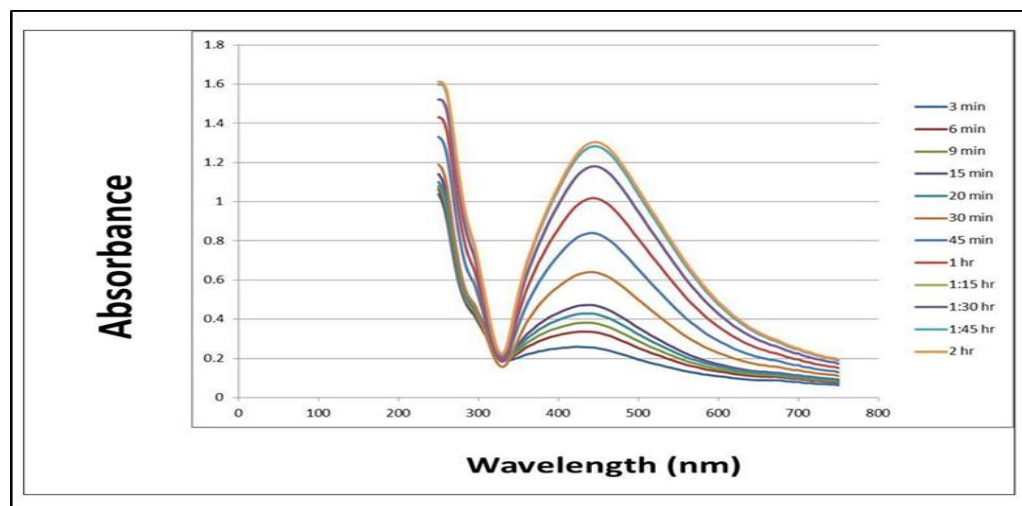
Silver nanoparticles were prepared from silver nitrate salt ( $\text{AgNO}_3$ ) and extract of Habaq (*Mentha longifolia*) leaves as reducing and capping agent for AgNPs. 250 mL from silver nitrate (0.01 M) was added to 25 mL of the Habaq plant extract and the volume was completed to 500 mL using distilled water then heated for 2 hours in  $60^\circ\text{C}$ . The silver nanoparticles were characterized by ultraviolet spectrophotometer (Thermo Scientific GENESYS 10S UV-Vis), X-ray diffraction (XRD) analysis (Shimadzu x-ray diffractometer XRD-6100) and transmission electron microscopy (TEM, model Joel-JEM 100CX, Japan).

The growth experiment was designed using four concentrations of AgNPs (0, 10, 50, 150 ppm) with three drought levels (80%, 50% and 30%). Sterilized grains were soaked for two hours in different concentrations of AgNPs then transferred to petri dishes and incubated in the dark for 24 hours at room temperature before sowing into pots. Seven pre-soaked grains were placed in each pot beneath the soil surface (1 cm depth). Pots were transferred to growth chamber at 25 C° (photoperiod 16 h, Humidity 70%) at laboratory of Faculty of Science, Taibah University, Saudi Arabia. Pots was irrigated with water (80% level) during the first four days, and from the fifth day 15 mL of AgNPs was added to irrigating water in order to keep constant drought levels during experimental period (14 day). After growth period, morphological parameters such as: shoot and root fresh and dry weights, shoot and root length were measured. The physiological measurements were determined, photosynthetic pigments according to Lichtenthaler *et al*, (1987), total carbohydrates according to Dubois *et al*, (1956), proline content according to Bates *et al*, (1973) and total antioxidant enzymes activity according to Malusa *et al*, (2006). Ultra-structure of *Zea mays* leaves was investigated using transmission electron microscopy. The data analyzed statistically using Minitab software version 18. The two-way ANOVA-test used to compare the significance and non-significance difference between the treatments according post-hoc Tukey.

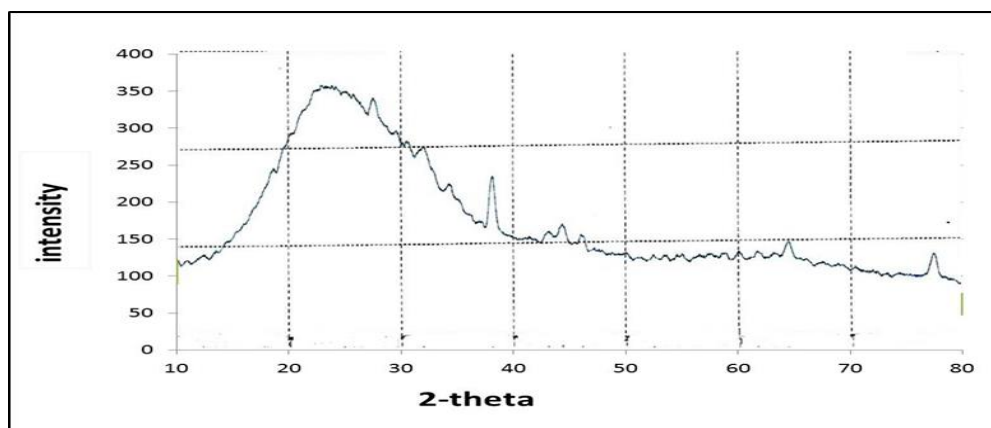
### 3. Results and discussion

The synthesized AgNPs from *Mentha longifolia* show maximum absorbance at 445 nm (Fig.1). This result was concordant with previous studies for green synthesis of AgNPs utilizing *Zingiber officinale* extract and gave the surface plasmon band around 430-450 nm (Shalaby *et al*, 2015). Other studies on synthesis of AgNPs from *Rosa rugosa* and *Ficus benghalensis* gave surface plasmon around 451-458 nm and band at 410 nm respectively (Dubey *et al*, 2010; Saxena *et al*, 2012). The XRD analysis of AgNPs from *Mentha longifolia* shown peak at  $2\theta = 38^\circ, 44^\circ, 64^\circ$ , and  $77^\circ$  reflected in the (111), (200), (220), and (311) planes (Fig. 2). Indicate face centered cubic AgNPs. Other studies were also found The formation of AgNPs by XRD analysis was explained by four peaks at  $38.11^\circ, 44.29^\circ, 64.45^\circ$  and  $77.39^\circ$  they are reflected to the (111), (200), (220), and (311) planes (Kumar *et al*, 2014; Shalaby *et al*, 2015; Anandalakshmi & Venugobal, 2017). Transmission Electron Microscopy was performed to investigate the size, shape and morphology of the AgNPs. Using *Mentha longifolia* extract indicated spherical in shape and particle sizes range between 2.45-16.85 nm (Fig. 3).

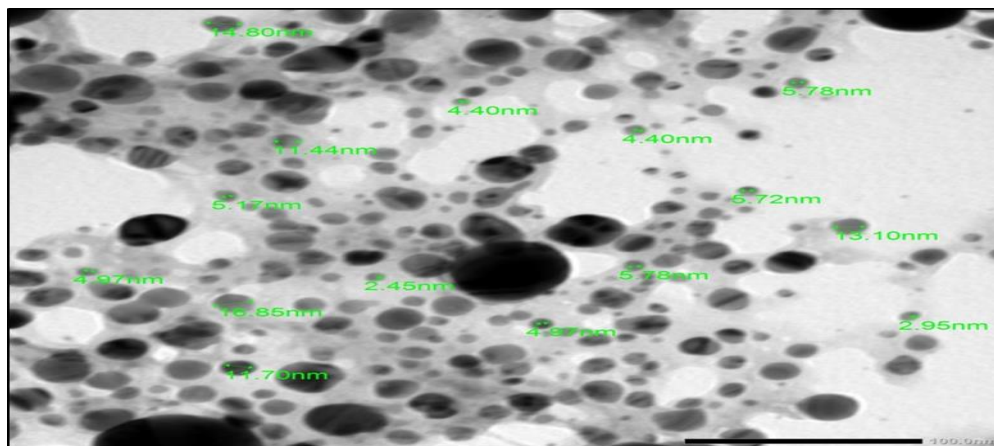
There is reported in literature TEM analyses of synthesized AgNPs from *Boerhaavia diffusa* was a spherical shape with an average size of about 25 nm (Kumar *et al*, 2014). Dwivedi & Gopal (2010) and Lakshmanan *et al*, (2018) used *Cleome viscosa* and *Chenopodium album* respectively found AgNPs also spherical, with range between 5-30 nm.



**Fig. 1:** UV/Vis absorption spectrum of different heating time effect (temp. = 60-65 °C).

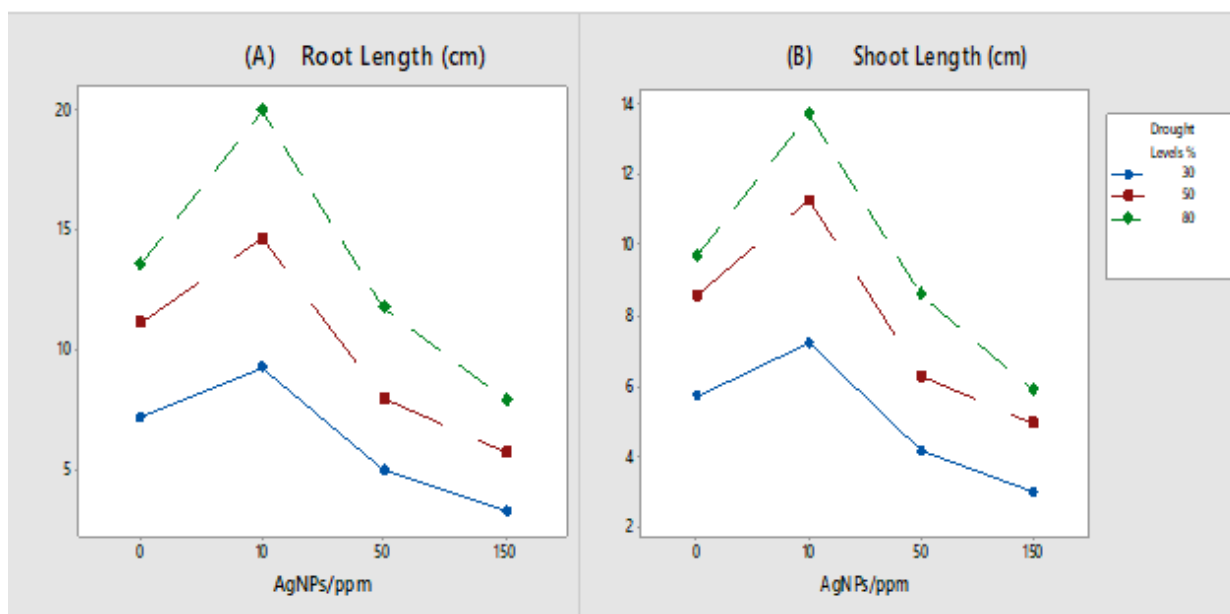


**Fig. 2:** XRD spectrum of silver nanoparticle prepared by Habaq mint extract.



**Fig. 3:** (TEM) analysis of silver nanoparticle prepared by Habag mint extract.

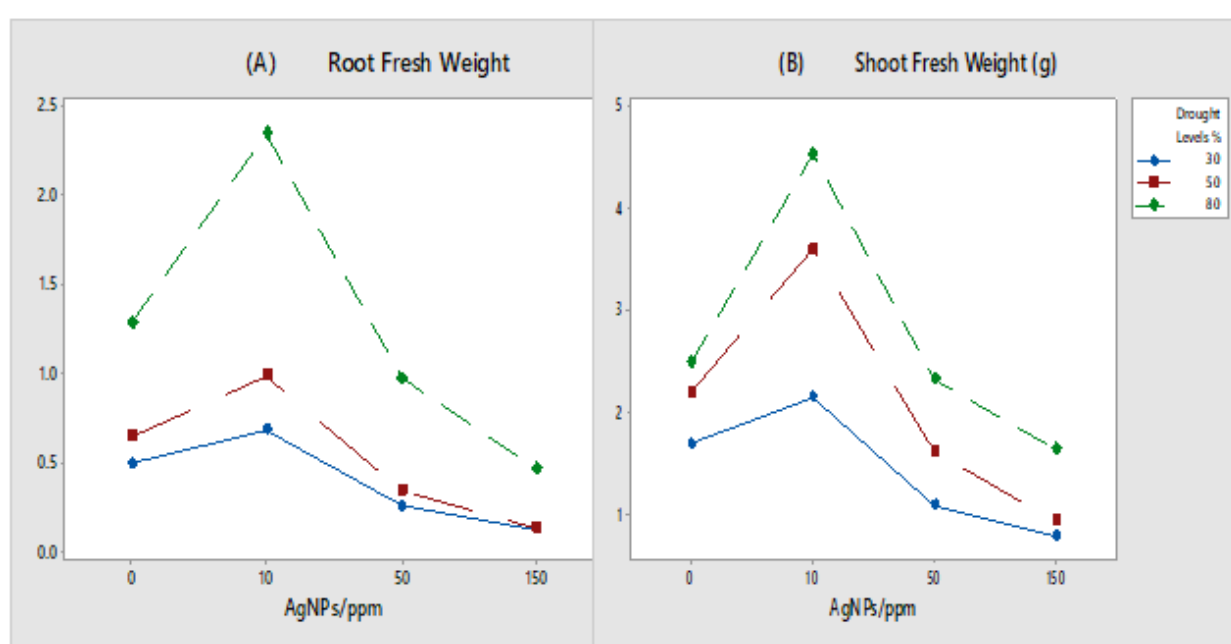
Water holding capacity is the amount of moisture the soil can retain. Plant growth is directly affected by the water holding capacity of the soil, and so is the organic matter content. Appropriate amount of water in the soil allows dissolution of nutrients, mineral and ions, making them bio-available for the plant. The highest root length was achieved from grains presoaked and irrigated with 10 ppm AgNPs under well-watered conditions; it reached 19.98 cm compared to 13.55 cm in the control. The lowest root length was recorded for 150 ppm of AgNPs; it reached 3.24 cm compared to 13.55 cm in the control. The same trend was recorded for the shoot length (Fig.4). This data was significant in indicating that growth of mechanical structures of the plant such as the root and shoots became independent of soil water holding capacity in the presence of AgNPs. However, the addition of higher concentration of AgNPs such as 150 ppm at all drought levels pushed the root length even below the control plant. This indicated that the plant promoting properties of AgNPs only sustained at lower concentrations and thus would be different for all treatments (Fig. 4). Similar results were reported by previous studies evaluating the growth parameters of rice by application of AgNPs (Thuesombat *et al*, 2014; Iqbal *et al*, 2017). This was also observed in a study conducted by Ashkavand *et al*, (2015) wherein treatment with 50 ppm of silica nanoparticles caused an increase in water holding capacity of the soil of plants put under stress.



**Fig. 4:** Effect of AgNPs (0, 10, 50 and 150 ppm) on A) root length, B) shoot length of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

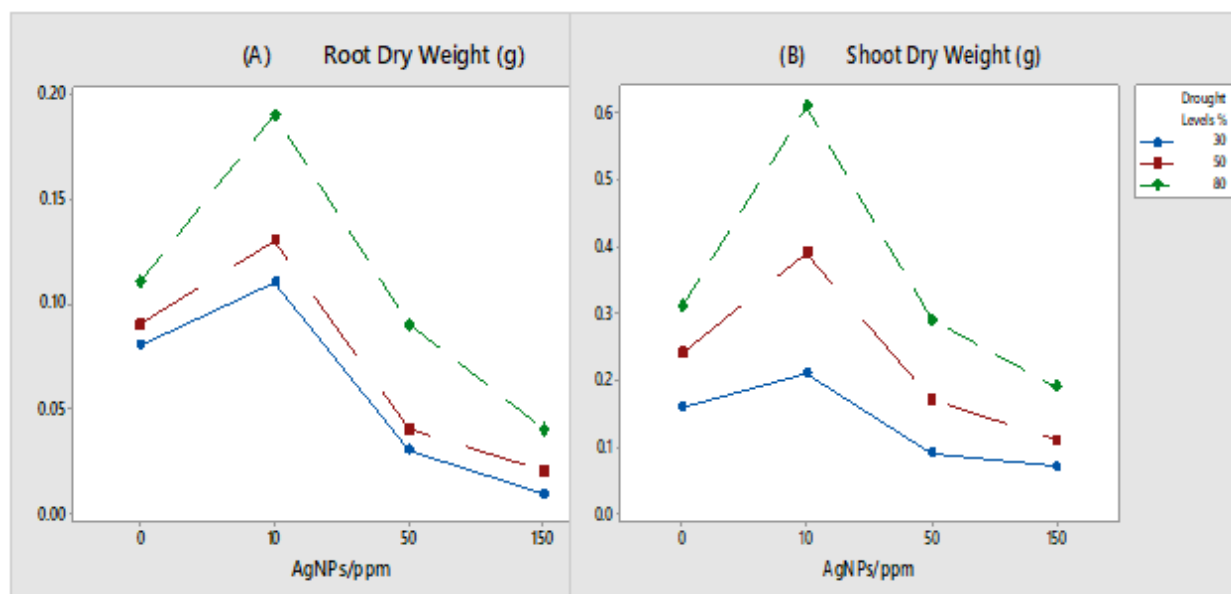
Data in (Fig. 5, 6) showed that fresh and dry weight seemed to be higher at 80% drought level followed by 50% then 30% levels. In overall, shoot fresh weight seemed to be high at all concentrations of AgNPs at 80% drought level. Different concentrations of AgNPs affect the shoot fresh & dry weight especially at 150 ppm AgNPs it was (1.64 g) and (0.19 g) respectively. The same trend was recorded for root fresh & dry weight. Combination of 30% drought level and 10 ppm AgNPs cause a significant increase in both fresh & dry weight of shoot of *Zea mays* seedlings (2.15 g, 0.21 g) compared to (1.69 g, 0.16 g) at 30% drought level only respectively. Previous studies reported that AgNPs have an effect on the increase the shoot and root length of *Brassica juncea* plant (Pandey *et al*, 2014). Silver nanoparticles effect on wheat plants were protected from heat stress and enhanced shoot length has been visualized compared to control treatment (Iqbal *et al*, 2017). The SiO<sub>2</sub> nanoparticles increased the morphological parameters on hawthorn seedlings under drought stress (Ashkavand *et al*, 2015).





**Fig. 5:** Effect of AgNPs (0, 10, 50 and 150 ppm) on A) root, B) shoot fresh weight of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

Three different pigments were extracted and quantified; chlorophyll *a*, *b* and carotenoids. It was found that application of 10 ppm of AgNPs enhanced the productivity of all three pigments and application of 150 ppm AgNPs inhibited their synthesis. The present results showed clearly that water stress had a negative effect on the chlorophyll contents and its components of maize. Decreased moisture level caused a decrease in chlorophyll content (Table. 1). Chlorophyll *a* and *b* decreased by water stress. The Strong reduction in chlorophyll *a* and *b* was observed mainly at the lowest water holding capacity (30%) with 150 ppm AgNPs as compared to control. The decrease in chlorophyll *a* was about 18.5% while it was about 38.3% for chlorophyll *b*. In fact, chlorophyll *a* with 30% drought level was reduced at 50 ppm ( $14.76 \text{ mg/g}^{-1}$ ) and at 150 ppm ( $13.46 \text{ mg/g}^{-1}$ ) while remains increased significantly at the lowest concentration 10 ppm AgNPs ( $18.68 \text{ mg/g}^{-1}$ ) compared to well-watered control. As well, carotenoids content was the highest at 10 ppm ( $7.24 \text{ mg/g}^{-1}$ ) and the lowest at 150 ppm ( $4.64 \text{ mg/g}^{-1}$ ). Exposure to high AgNPs concentrations had retardation on total chlorophyll content in *V. radiata* and *B. campestris* when compared to control treatment (Mazumdar, 2014). Farooq *et al* (2009) cited cell enlargement is affected more by drought stress than cell division as water stress leads to a decline in the overall growth of a plant as it leads to reduction in various biochemical and physiological process of a plant which includes respiration, ion intake, photosynthesis, nutrient metabolism, and growth promotion among others.



**Fig. 6:** Effect of AgNPs (0, 10, 50 and 150 ppm) on A) root, B) shoot dry weight of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

A change in total soluble sugars at 80% drought level was irregular, by addition of 10 ppm AgNPs it increased in the roots and the shoots of the examined plant. On the other hand, treatments with 150 ppm AgNPs at 30% drought level decreased the total soluble sugars content of roots by 40.7% and increased in the shoot by 3.8% with respect to the control value the 30% drought level only (Table. 2). Furthermore, seedling grown under 30% drought level resulted in an increase of total soluble sugar by (1.11 $\mu$ g/g DWT) in roots at 10 ppm AgNPs whereas; it decreased (2.71 $\mu$ g/g DWT) in the shoots when compared with well-watered maize seedlings respectively. Conversely, at 150 ppm AgNPs total soluble sugar of shoot increased (3.39 $\mu$ g/g DWT) and root total soluble sugar decreased (0.48 $\mu$ g/g DWT) compared to control. This application of AgNPs with simultaneous monitoring of plant sucrose levels can help combat drought stress in plants and make them tolerant (Nemeskeri *et al*, 2015; Pingping *et al*, 2017). The same results were found on the effect of low AgNPs concentrations in the fenugreek plant increased the total carbohydrate content (Sadak, 2019). In case of cotton plant has been reported the effect of TiO<sub>2</sub> and SiO<sub>2</sub> nanoparticles under drought stress, increased the shoot total carbohydrate content (Shallan *et al*, 2016).

**Table 1:** Effect of AgNPs (0, 10, 50 and 150 ppm) on chlorophyll *a*, chlorophyll *b* and carotenoids of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

| Drought Levels% | AgNPs/ppm | chlorophyll <i>a</i> (mg/g <sup>-1</sup> ) | chlorophyll <i>b</i> (mg/g <sup>-1</sup> ) | Carotenoids (mg/g <sup>-1</sup> ) |
|-----------------|-----------|--|--|-----------------------------------|
| 80              | 0         | 25.62± 0.28 ab                             | 8.72± 0.32 b                               | 9.61± 0.33 b                      |
|                 | 10        | 28.61± 0.23 a                              | 11.87± 0.1 a                               | 13.14± 0.33 a                     |
|                 | 50        | 23.23± 0.14 b                              | 7.55± 0.2 d                                | 7.93± 0.34 d                      |
|                 | 150       | 19.56± 0.26 c                              | 6.19± 0.07 fg                              | 6.84± 0.12 ef                     |
| 50              | 0         | 19.18± 0.1 cd                              | 6.46± 0.16 d                               | 7.70± 0.15 d                      |
|                 | 10        | 22.42± 0.16 c                              | 7.39± 0.42 c                               | 9.76± 0.21 c                      |
|                 | 50        | 17.91± 0.03 de                             | 5.27± 0.3 fg                               | 6.52± 0.18 ef                     |
|                 | 150       | 14.84± 0.13 f                              | 4.27± 0.14 h                               | 5.40± 0.24 g                      |
| 30              | 0         | 16.52± 0.14 ef                             | 5.66± 0.28 ef                              | 6.43± 0.24 f                      |
|                 | 10        | 18.68± 1.1 e                               | 6.28± 0.14 de                              | 7.24± 0.07 de                     |
|                 | 50        | 14.76± 0.53 f                              | 4.57± 0.23 gh                              | 5.46± 0.3 g                       |
|                 | 150       | 13.46± 1.02 g                              | 3.49± 0.28 i                               | 4.64± 0.24 h                      |

Note: Means that do not share a letter are significantly different.

**Table 2:** Effect of AgNPs (0, 10, 50 and 150 ppm) on shoot and root total soluble sugar of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

| Drought Levels % | AgNPs/ppm | Shoot Total Soluble Sugar (µg/g DWT) | Root Total Soluble Sugar (µg/g DWT) |
|------------------|-----------|--------------------------------------|-------------------------------------|
| 80               | 0         | 1.52±0.21 fg                         | 1.52 ±0.19 ab                       |
|                  | 10        | 1.65±0.3 f                           | 1.81±0.12 a                         |
|                  | 50        | 1.36±0.17 gh                         | 1.48 ±0.14 ab                       |
|                  | 150       | 1.09±0.23 h                          | 1.19 ±0.15 bc                       |
| 50               | 0         | 1.91±0.08 ef                         | 1.02 ±0.09 bc                       |
|                  | 10        | 2.09±0.45 de                         | 1.55 ±0.6 ab                        |
|                  | 50        | 2.28±0.1 d                           | 0.88 ±0.19 c                        |
|                  | 150       | 2.53±0.07 cd                         | 0.67 ±0.51 cd                       |

|           |     |              |               |
|-----------|-----|--------------|---------------|
| <b>30</b> | 0   | 3.26±0.08 bc | 0.81±0.06 c   |
|           | 10  | 2.71±0.4 c   | 1.11±0.54 bc  |
|           | 50  | 3.01±0.09 ab | 0.74 ±0.1 cd  |
|           | 150 | 3.39±0.21 a  | 0.48 ±0.11 cd |

Note: Means that do not share a letter are significantly different.

Proline is an osmoprotectant that has been shown to accumulate in plants in response to stress. Result in table 3 showed that proline content increased in response to water stress by 25.6 and 12.5 fold of the control values in the roots and shoots of the examined maize seedlings. Grains pretreated with 10 ppm AgNPs led to increase in the concentrations of proline in the roots (0.15µg/g FWT) and shoots (0.11µg/g FWT) with respect control. Proline contents increased the roots by 1.2 fold; the corresponding value for shoot was 1.4 fold under 30% drought level and 10 ppm AgNPs with respect to the 30% drought level. Proline content increased by 1.7 and 2.8 fold in roots and shoots under 30% drought level at 150 ppm AgNPs with respect to the 30% drought level respectively. The application AgNPs at such stage will also aid the plant in synthesizing more proline and assist in drought stress (Keyvan, 2010; Jayant & Sarangi, 2014). Previous study conducted with zinc nanoparticles has also revealed similar results (Hashemi *et al*, 2018). The same results were found in peanut plant under drought stress total proline content increased significantly in all tested varieties (Jayant & Sarangi, 2014). Proline accumulation was increased in sugarcane plant under drought (de Oliveira *et al*, 2018).

**Table 3:** Effect of AgNPs (0, 10, 50 and 150 ppm) on shoot and root proline of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

| Drought Levels % | AgNPs/ppm | Shoot Proline (µg/g FWT) | Root Proline (µg/g FWT) |
|------------------|-----------|--------------------------|-------------------------|
| <b>80</b>        | 0         | 0.1±0.01 g               | 0.11±0.27 fg            |
|                  | 10        | 0.11±0.3 g               | 0.15±0.01 f             |
|                  | 50        | 0.15±0.02 fg             | 0.25±0.14 f             |
|                  | 150       | 0.21±0.13 fg             | 0.63±0.03 ef            |
| <b>50</b>        | 0         | 0.24±0.02 fg             | 0.92±0.43 d             |
|                  | 10        | 0.29±0.06 fg             | 1.52±0.09 e             |
|                  | 50        | 0.50±0.1 ef              | 2.21±0.44 cd            |

|           |     |                    |                    |
|-----------|-----|--------------------|--------------------|
|           | 150 | $0.77 \pm 0.16$ de | $2.42 \pm 0.36$ cd |
| <b>30</b> | 0   | $1.25 \pm 0.37$ c  | $2.82 \pm 0.21$ b  |
|           | 10  | $1.76 \pm 0.17$ d  | $3.41 \pm 0.3$ bc  |
|           | 50  | $2.85 \pm 0.04$ b  | $4.23 \pm 0.14$ a  |
|           | 150 | $3.5 \pm 0.2$ a    | $4.95 \pm 0.46$ a  |

Note: Means that do not share a letter are significantly different.

Free radical scavenging capacity increased in the roots and shoots by increasing tested AgNPs concentration compared to control value. It is noteworthy to mention that extracted roots and shoots that treated with AgNPs at 80% drought level had higher scavenging activity than control. For example, it recorded 7.67, 8.27 and 9.59  $\mu\text{g/ml}$  FWT in roots under 10, 50 and 150 ppm AgNPs respectively. The corresponding values for shoots were 4.64, 6.34 and 7.16  $\mu\text{g/ml}$  FWT respectively. Under the interaction between AgNPs and water stress tested organs had higher scavenging activity than control. DPPH' scavenging activity significantly different in roots and shoots of the maize seedlings as compared with the stressed value (30% only), it increased by about 33.91  $\mu\text{g/ml}$  FWT and 25.16  $\mu\text{g/ml}$  FWT under 30% drought level with 150 ppm AgNPs (Table. 4). Application of 150 ppm of AgNPs stimulated the production of antioxidants further generating stress tolerant conditions in the plant. Application of zinc nanoparticles to soybean have also shown to increase the antioxidant content (Hashemi *et al*, 2018). Other studies reported drought stress; increase the total antioxidant capacity of three plants from the genus *Juncus* (Al Hassan *et al*, 2017). A significant increase in the activity of total antioxidant was reported in sugarcane plant affected by drought stress (de Oliveira *et al*, 2018). Different antioxidant enzymes of *Lemna gibba* plant were significantly affected by high concentrations of AgNPs (Varga *et al*, 2019).

**Table 4:** Effect of AgNPs (0, 10, 50 and 150 ppm) on shoot and root total antioxidant capacity of *Zea mays* seedlings under different drought levels (80, 50 and 30%).

| drought level % | AgNPs/ppm | Shoot Total Antioxidant Capacity ( $\mu\text{g/ml}$ ) | Root Total Antioxidant Capacity ( $\mu\text{g/ml}$ ) |
|-----------------|-----------|---|--|
| <b>80</b>       | 0         | $3.74 \pm 1.21$ h                                     | $5.49 \pm 1.11$ h                                    |
|                 | 10        | $4.64 \pm 1.22$ h                                     | $7.67 \pm 1.06$ gh                                   |
|                 | 50        | $6.34 \pm 1.4$ gh                                     | $8.27 \pm 1.42$ fgh                                  |
|                 | 150       | $7.16 \pm 1.78$ gh                                    | $9.59 \pm 1.33$ fgh                                  |

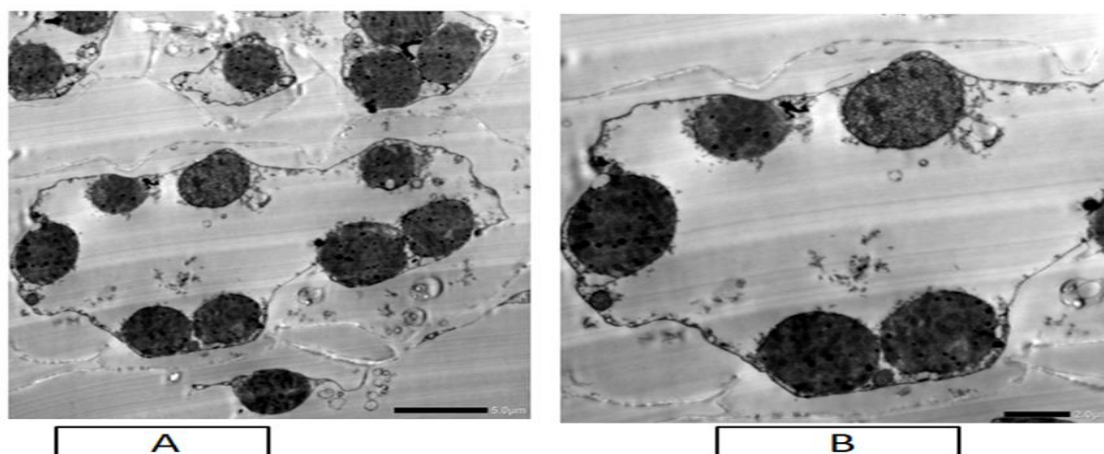
|           |     |                |                 |
|-----------|-----|----------------|-----------------|
| <b>50</b> | 0   | 8.94± 0.76 fg  | 11.31± 1.78 efg |
|           | 10  | 10.79± 0.95 ef | 12.5± 2.04 ef   |
|           | 50  | 13.63± 1.03 de | 15.53± 0.6 de   |
|           | 150 | 18.57± 1.42 bc | 22.27± 2.46 bc  |
| <b>30</b> | 0   | 13.51± 0.6 de  | 16.73± 1.1 d    |
|           | 10  | 15.7± 1.33 cd  | 18.62± 0.44 cd  |
|           | 50  | 20.71± 1.36 b  | 25.59± 0.59 b   |
|           | 150 | 25.16± 0.82 a  | 33.91± 1.79 a   |

Note: Means that do not share a letter are significantly different.

As shown in figure 7, after 14 days of 30% drought level treatment, the ultrastructure of *Zea mays* chloroplasts changed significantly. Under water stress, grana thylakoid lamellae were compressed, chloroplasts were severely deformed into irregular shapes, without starch granules accumulated. In addition, water stress induced a separation between cell membranes and chloroplasts. Further observation of the structure of the water-stressed thylakoid membrane revealed that the grana thylakoid lamellae were disordered and the stroma thylakoid lamellae were fractured. We observed that ultra-structure of leaf treated with 10 ppm AgNPs at 30% drought level was less shrinking of the chloroplast than 0 ppm AgNPs at 30% drought level; the chloroplasts appear oval in shape and migrated towards the plasma membrane (Fig 8).

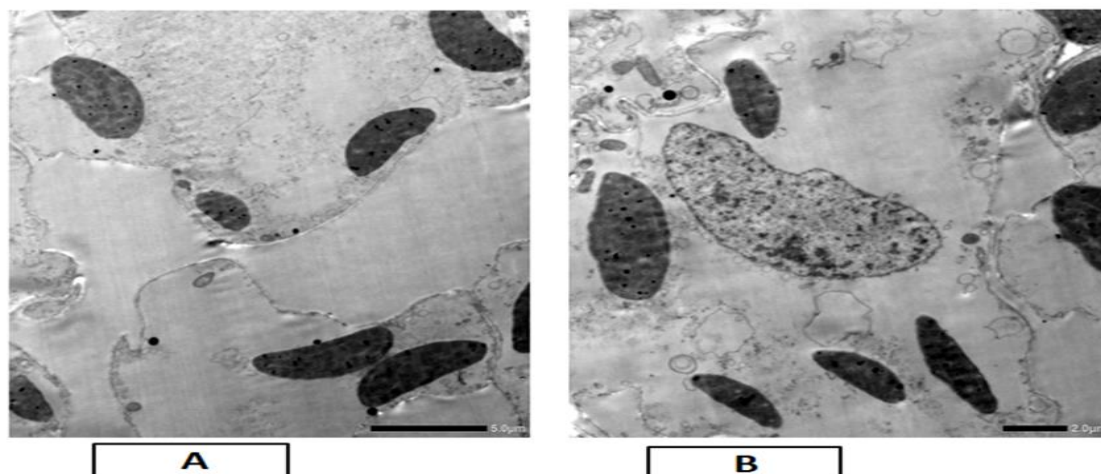
In figure 9, destroyed cell wall and chloroplast was devastated due to drastic effect of 150 ppm AgNPs at 30% drought level, cell membrane plasmolyzed and became away from the cell wall. Generally, there is a total destruction in all cell components at this treatment. In this study, the drought stress and AgNPs caused some destruction of the cell and organelle ultra-structure depends on their concentration. The chloroplast disturbances observed were similar to other previous study results. In *Triticum durum* plant under drought stress indicated folded cell wall, a plasma membrane with invaginations, Thinning and partial tearing of the chloroplast envelope was also observed and significant damage in the mesophyll chloroplasts and disordered thylakoid membranes (Assem *et al*, 2017). Also, drought stress negatively affected the chloroplasts and mitochondria of *Triticum aestivum*.

In addition, membrane permeability and stroma acidification increase under stress and contribute to chloroplast enzyme inhibition (Grigorova *et al*, 2012). Mazumdar (2014) observed results, changes in the cellular organization in high concentrations of AgNPs treated the *Vigna radiata* and *Brassica campestris* plants resulted clear alteration in the leaf cellular ultra-structure was detected to deposition of AgNPs and aggregate particle was found inside the cell wall and the chloroplast was shrinking in their size. Aleksandrowicz-Trzcińska *et al*, (2019) revealed that there was no negative change in the ultra-structure of *Quercus robur* plant treated with low concentrations of 5 ppm AgNPs. The presence of the AgNPs in cell wall and the chloroplast enlarged size of the vacuoles, large grains of starch with more numbers of plastoglobules appearing in chloroplasts and with a change in the shape of the latter from lenticular to spherical with osmophilic globules were detected.

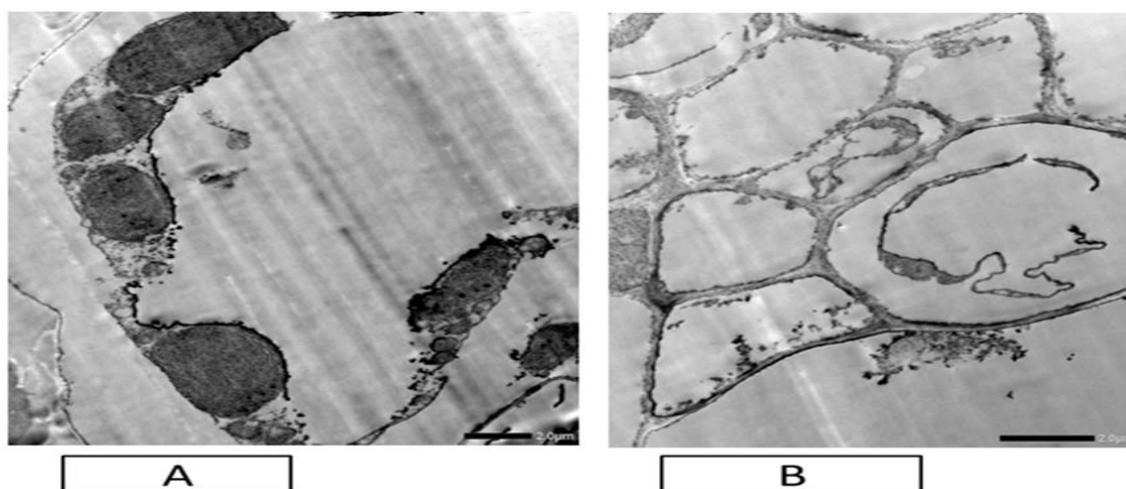


**Fig. ۷:** Transmission electron micrographs of *Zea mays* seedling leaf ultra-structure of 0 ppm AgNPs with 30% drought level. The magnification of panel A 1200X; the magnification of panel B is 2000X.





**Fig. 8:** Transmission electron micrographs of *Zea mays* seedling leaf ultra-structure of 10 ppm AgNPs with 30% drought level. The magnification of panel A 1500X; the magnification of panel B is 2000X.



**Fig. 9:** Transmission electron micrographs of *Zea mays* seedling leaf ultra-structure of 150 ppm AgNPs with 30% drought level. The magnification of panel A 2000X; the magnification of panel B 3000X



#### 4. Conclusion

The present study was conducted application of 10 ppm AgNPs was able to stimulate the lengths and weights of the *Zea mays* seedling. Also, help the plant under 30% drought level to improve the physiological functions of the plant, unlike other concentrations. The results of this work show potential negative or positive effect of AgNPs depend on the concentrations. The approach might be useful in countries often suffering from drought climate to enhance the plant growth in drought stress conditions.

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