# Influence of spraying Nano-curcumin and Nano-glycyrrhizic acid on resistance enhancement and some growth parameters of soybean (Glycine max) in response to *Tetranychus urticae* infestation and drought stress

**Azza M. Salama**
Cairo University Faculty of Agriculture
**Hala H. Alakhdar**
Agricultural Research Center
**Tahsin Shoala** ( Tahsinshoala2000@gmail.com )
Misr University for Science and Technology https://orcid.org/0000-0002-5659-7454

**Research Article**

**Keywords:** soybean, Nano-curcumin, Nano-glycyrrhizic acid, Tetranychus urticae, drought, anatomy, growth parameters

**Posted Date:** July 21st, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-651213/v1

# Abstract

This study was carried out during two successive seasons 2018 and 2019, on soybean plants (Glycine *max L*. cv. Giza 90) atQaha Research Station, Plant Protection Research Institute, Qalyoupia governorate, Egypt. In order to investigate the effect of curcumin and glycyrrhizic acid ammonium salt (GAS-NPs) as a foliar application under water deficit on growth, yield, anatomical and chemical parameters. The obtained results revealed that, under water deficit over seasons significantly decreased all morphological and yield characters. Glycyrrhizic acid ammonium salt nanoparticles gave the highest averages of plant height, branches and leaves number and fresh and dry weight of plant at 1 mM. Moreover, number of pods, 100 seed weight and seed yield (kg/ha) increased significantly as a result of spraying with glycyrrhizic acid ammonium salt nanoparticles under water deficit. As to anatomical studies, water deficit decreased the values of the leaf and stem anatomical parameters. Treatment with curcumin or glycyrrhizic acid ammonium salt nanoparticles at 50% of water holding capacity increased the thickness of mid vein and xylem and phloem tissues. Likewise, such treatment increased stem diameter due mainly to the increase in the thickness of cortex, phloem and xylem tissues compared with control.


# Introduction

Soybean (*Glycine max* (L.)) is a worldwide essential plant, a native of China, where it has been part of the diet for several thousand years. More in recent times, soybeans have become widely used in the Eastern diet, being used to produce a range of ingredients: soya-flour and soya-protein, which are used to make foodstuffs and vegetarian alternatives. Soybean is used as an emulsifier in many foods; and soya milk, a vegan alternative to cow's milk, infant formulas, and a valuable contribution to nutrient intakes and may as well have several potential health advantages. It is principally sensitive to the water deficiency during the blooming process, the legume and seed growing process, whereas leads to a reduction in the seed size and yield production (Georgieva and Nikolova 2019).

Climate change is expected to primarily affect precipitation, temperature and potential evapotranspiration, and, thus, is likely to affect the occurrence and severity of meteorological droughts. An important question for the assessment of future impacts (i.e. socio-economic and environmental) is how changes in meteorological drought will affect soil water drought and hydrological drought, i.e. groundwater and streamflow droughts. Soil water drought is, for example, relevant for agriculture, terrestrial ecosystems, and health through the occurrence of heatwaves, whereas hydrological drought has significance for among others water resources (agriculture, domestic and industrial water use), aquatic ecosystems, power generation, and navigation (Georgieva and Nikolova 2019).

Drought is a sustained and regionally extensive occurrence of below-average natural water availability. It is mainly caused by low precipitation and high evaporation rates, but in regions with a cold climate, temperatures below zero can also give rise to winter drought. Drought can be characterized as a deviation from normal conditions in the physical system (climate and hydrology), which is reflected in variables such as precipitation, soil water, groundwater and stream flow. Drought is a recurring and worldwide phenomenon having spatial and temporal characteristics that vary significantly from one region to another. Drought should not be confused with aridity, which is a long-term average feature of a dry climate, or with water scarcity, which reflects conditions of long-term imbalances between available water resources and demands. It is important, however, to note that the most severe human consequences of drought are often found in arid or semi-arid regions where water availability is already low under normal conditions (aridity), demand is close to, or exceeds, natural availability and society seldom lacks the capacity to mitigate or adapt to drought (Nikolova et al., 2014)

There have been diverse hypotheses about the consequences of drought stress on the development and reproduction of the two spotted spider mites *Tetranychus urticae* Kosh, (Acari: Tetranychidae). Drought stress causes an increase in mite numbers and plant damage, besides their dietary activity stress to affect the density of spider mite and number of eggs-laid/ female. *T.urticae* is a highly polyphagous pest spreading rapidly, and notorious for developing resistance to pesticides. It is a critical pest on soybean, causes a significant reduction in yield. Under stress (biotic or abiotic) conditions, the plants present a series of changes affecting their growth and productivity. Only a few experiments considered the interactions between one of the main pests–spider mites and crops' water regime (Georgieva and Nikolova 2019; **Aalakhdar 2020**).

The application of Nanotechnology is studying the new physical properties of different materials in the nanosize. Nanomateriales in the nanosize have changed the vision of the world towards physical structure and size of the same material. Nanomaterials have altered the direction of the biological research towards nanoscience and became one of the most important and applicable sciences in different biological aspects. Application of nanomaterials in biological research decreased pre- and postharvest diseases; and decreased the pollution in the environment. Different materials in the normal size could be toxic so that converting those materials to nanosize will change the physical properties and increase the level of toxicity. Converting natural and safe products to nanosize could play dual role in the nanoscience by enhancing the nanoactivity of natural products against different pathogens, increasing shelf life fruits and vegetables in room temperature, decreasing the side effect and toxicity of using pesticides, curing different human diseases and decreasing the negative or bad side of using unsafe nanomaterials. Application of nanotechnology could take a positive or negative impact according to the first choice of your material.

Curcumin in turmeric are mainly amassed in Turmeric rhizomes (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the Zingiberaceae family. Turmeric is principally applied as a treatment for inflammatory conditions and in traditional Chinese medicine; it is used as stimulant, anti-bacteria, aspirant, carminative, cordials, astringent, detergent and diuretic, (Remadevi et al., 2007; Sasikumar 2005**).**

Glycyrrhizic acid (GA) is considered as one of the major bioactive elements in licorice, which developed from rhizomes and roots of *Glycyrrhiza glabra*. Licorice has been comprehensively consumed as herbal medicine against many diseases. Also, Glycyrrhizic acid can be utilized in the form of Glycyrrhizic acid ammonium salt **(IHIDMA 2002).**

This investigation was occurred to study the response of soybean plant to the application of curcumin nanoparticles and Nano-glycyrrhizic acid under drought condition and its relation to the population of the two-spotted spider mite *Tetranychus urticae*.

## Material And Methods

Field experiments were carried out on an experimental farm at Qaha Research Station, Plant Protection Research Institute, Qalyoupia governorate, Egypt during the 2018 and 2019 seasons on soybean, (*Glycine max* L. Merrill). Seeds of soybean were secured from Field Crops Institute, Agricultural Research Center, Egypt. Soybean seeds were sown on the first week of May in both seasons. The experimental design was a randomized complete block (RCBD) with three replications. Each plot consisted of six ridges, 70 cm apart and four m long. Seeds were planted at density of 20 plants per a meter of a linear ridge. All other agricultural practices were conducted as recommended for Qaha location (**Alakhdar *et al.*, 2016**). Three seeds grown per hill, after 25 days from sowing, the plants were thinned to one plant/hill.

#### Synthesis of Nano curcumin

Curcumin was purchased from Sigma-Adrich Company (CAS Number: 458-37-7), Stock of curcumin solution (5 mg/ml) was prepared by dissolving curcumin powder in Dichloromethane (20 ml). One ml of stock solution was added to boiling water (50 ml) in drop-wise manner under ultra-sonication condition (XUBA3Analogue Ultrasonic Bath, Grant Company) with an ultrasonic power and frequency of 50 kHz. The solution was sonicated for about 30 min. After sonication, the mixture was stirred at 800 rpm for 20 min till the orange colored precipitate was obtained. Thereafter, supernatant was discarded and the pellet obtained was used for further study (**Hammam and Shoala 2020**).

**Glycyrrhizic acid ammonium salt nanoparticles Preparation**

Glycyrrhizic acid ammonium salt purchased from Sigma-Adrich company (CAS number : 53956-04-0), 0.1 mg of Glycyrrhizic acid Ammonium salt was dissolved in 1 ml absolute ethanol at room temperature (20-25°C), then stored in the fridge (4°C) (**Abdel-Rahman *et al.*, 2020**).

**Characterization of nanomaterials by using Dynamic light scattering (DLS)**

Measurement of Glycyrrhizic acid ammonium salt (GAS-NPs) and Curcumin nanoparticles (Cu-NPs) distribution and size was performed by a dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. Prior to measurement, 30µl of the nanoparticles were diluted with 3ml of water at 25°C. Particle size data was expressed as the mean of the Z-average of 3 independent batches of the nanoparticles.

**Treatments**

The treatments were control at 100% FC; control at 50% FC, curcumin at 0.5 ml/l; curcumin at 1.0 ml/l; glycyrrhizic acid at 0.5 ml/l and glycyrrhizic acid at 1.0 ml/l. A field capacity was calculated under dripping irrigation when zones of water overlap each other on the line. The treatments were applied twice; at 30 and 45 days after sowing. Random samples were taken from each plot at 60 days after sowing to record the morphological characters. At harvest (120 days), samples were randomly taken from each pot to determine the yield characters. All plants received recommended dose of NPK fertilizers.

**The seasonal density of *T. urticae* on soybean after treatment with curcumin nanoparticles and glycyrrhizic acid under drought and normal irrigation:**

The seasonal density of spider mite, *T. urticae* was determined in plots of different treatments from the last week of May to the first week of October during the two seasons 2018-2019. Ten plant leaves were selected from each replicate fortnightly, the number of *T. urticae*, was counted by the aid of a stereomicroscope at Acarology Lab. The mean density of total life stages (immature and adults) was statically analyzed using analysis of variance (ANOVA) and assessed among all treatments of soybean to evaluate the mean reduction percentage and variations in the different treatments and water irrigation mode on the total accumulation number of *T. urticae* (**Alakhdar *et al.*, 2015**).

**The fertility of *T. urticae* females on treated soybean leaves under drought stress**

Fifteen pot were conducted at Acarology Lab., Plant Protection Research Institute for each irrigation mode, the two watering frequency (T1– weekly irrigation, T2– two-week irrigation) applied to soybean as a host plants of *T. urticae*. Curcumin nanoparticles (3 and 6 mol.) and Nano-glycyrrhizic (1 and 3 mol.) were sprayed after 30 and 45 days of sown, three-pot replicates don't spray as a control. Treated leaves were collected from each plant for study the fertility and population buildup of *T. urticae* under laboratory conditions *in-vitro* conditions at 27±2ºC, 70±5% relative humidity for seven days. In this regard, one female was selected from the stock culture and transferred to a fresh leaf disc of each treated plants. Fresh leaf discs were made which were square or circular, and placed on the cotton bed in a petri-dish plate facing under surface upward. The cotton bed was kept wet by soaking with water twice daily so that the leaf discs remained fresh. Twenty-four hours later, the eggs laid were counted on these leaf discs and removed (**Poovizhiraja *et al.,* 2017**).

### Anatomical studies

A microscopical study was carried out to investigate the anatomical structure of the leaf and stem of soya bean plant, represented by the 4$^{th}$ internodes counted from the plant tip and the leaf was taken from the 4$^{th}$ internode represented by the middle of the lamina including the midrib at the age of 90 days.

Specimens were killed and fixed for at least 48 hours in F.A.A (10 ml formalin, 5 ml glacial acetic acid, 50 ml ethyl alcohol 95%, 35ml distilled water). Plant materials were washed in 50% ethyl alcohol and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax (melting point 52-54 °C). Transverse sections, 20μ thick, were cut using a rotary microtome and stained with double crystal violet/erythrosine combination and mounted in Canada balsam (**Nassar and El-Sahhar, 1998**). Measurements (μm) of the different tissues were taken, and averages of ten readings from five slides were calculated using a micrometer eye piece and micrometer stage. The slides were microscopically examined and photomicrographed at Botany Department, Faculty of Agriculture, Cairo University, Egypt.

### Chemical studies

### a. Mineral elements content in leaves

At the vegetation growth stage (60 Days) during the second season leaves from mature plants were subjected to determine total nitrogen (N), then calculated crude protein, by multiplied it with 6.25. Phosphorus (P), Potassium (K) and total chlorophyll were determined and calculated as % of dry weight according to **(A.O.A.C., 1999),** at Faculty of Agriculture, Cairo University Research Park (CURP).

### b. Determination of amino acids in seeds

At the harvest time during the second season samples from mature dried seeds were subjected to determine amino acids according to the methods described by **Csomos and Simon-Sarkadi, 2002; Shalabia,** and measured using Amino Acid Analyzer (AAA 400 INGOS Ltd) at Faculty of Agriculture, Cairo University Research Park (CURP).

### Statistical analysis

All collected data were subjected to statistical analysis as proposed by **Gomez and Gomez (1984)** and means were compared by LSD at 5% level of probability.

## Results

### Dynamic light scattering (DLS)

Measurement of curcumin and glycyrrhizic acid distribution and size were performed by a dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. Prior to measurement, 30μl of the nanoparticle was diluted with 3ml of water at 25°C. Particle size data were expressed as the mean of the Z-average of 3 independent batches of the nanoparticles. Dynamic light scattering (DLS) Dynamic light scattering technique was performed to understand the size distribution and the stability of prepared curcumin and glycyrrhizic acid nanoparticles showed that the size distribution range mainly within 16- 30 nm and 5-9 nm respectively as shown in (Fig 1 and Fig 3). The zeta potential is a key indicator of the stability of colloidal dispersions. So, colloids with high zeta potential (positive) are electrically stabilized (Fig 2 and Fig 4).

### The seasonal abundance of *Tetranychus urticae* (Kosh):

The soybean' mode of irrigation had a significant influence on the development and spider mite population density. Plants under water stress conditions were associated with a significantly increasing number of *T. urticae* mites. This result was observed as early as the first of plant exposure to drought. As indicated in table (1), the mean accumulated number of mites infested soybean was 982 was 19.76 % higher on the average than the density under irrigation 788 individuals, the differences were statistically significant. As illustrated in Fig. (5a and 5b), after treatment with curcumin nanoparticles, differences in the density of spider mite, *T. urticae*, were observed between the two used concentrations compared to the control in the two modes of irrigation. The mean percentage of reduction was 31 & 33.9% and 20.94 & 28.3% under drought and normal irrigation, respectively in the two studying seasons. Obtained results indicated that treated plants with 3 mM of glycyrrhizic acid ammonium salt nanoparticles harboured a significantly lower number of *T. urticae* than the control in the two seasons and irrigation methods. The average of reduction percentage of *T. urticae* was 39 and 33.11% compared with the control, respectively.

**Tabale (1): Mean accumulative number of *T.urticae* on soybean in response to curcumin and glycyrrhizic acid nanoparticles during the successive growing seasons of 2018-2019 under normal and drought stress.**

| Drought | Control | CU-NPs 3 mM | CU-NPs 6 mM | Control | GA-NPs 1 mM | GA-NPs 3mM |
|---|---|---|---|---|---|---|
| 50 % | 982±9.79$^{a}$ | 678±4.9$^{b}$ | 649±7.35$^{c}$ | 1025±20.41$^{a}$ | 693±13.47$^{b}$ | 626±20.41$^{c}$ |
| Reduction % | - | 31% | 33.9% | - | 32.43% | 39% |
| 100 | 788±14$^{a}$ | 623±9.39$^{b}$ | 565±12.66$^{c}$ | 743±7.48$^{a}$ | 572±12.25$^{b}$ | 497±8.16$^{c}$ |
| Reduction % | 19.76% | 20.94% | 28.3% | 27.52% | 23.02% | 33.11% |

Means followed by the same letter (s) are not significantly differed by the least significant Difference (Duncan, 1955)

### The fertility of *T. urticae* females on treated soybean leaves under drought stress

In our experiment, soybean water status affected the abundance of egg-laid per day/ mite's adult females. The intensity of increase in the number of mites on plants exposed to water deficit was significantly more pronounced throughout the test period, and it reached a 22.38% increase (on the 7th day). The performance of mites was decreased when reared on both nano curcumin-treated normal, and drought-stressed soybean plants, females laid a lower number of eggs than on control plants at 26.85& 32.57% and 28.55 and 35.44 for the two concentrations, 3 and 6 mM, respectively. The same trend was observed under treatment with glycyrrhizic acid nanoparticles, but the differences among treatments were less pronounced in the case of 3mM conc. about 37.39 and 40% in normal and drought conditions of irrigation (Fig 6a and 6b).

### Anatomical studies

Results in Tables (2 and 3) revealed that drought stress (50% FC) caused significant decreases for all anatomical characters for leaf and stem compared with normal irrigation (100% FC). Interestingly, treatments with nanoglycyrrhizic acid and nanocurcumin enhance the anatomical characters of leaves (Tables 2, 3).

**Table (2): Effect of nanocurcumin and Nanoglycyrrhizic acid under drought stress on anatomical characters of soybean leaf.**

| | Anatomical characters of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Mid vein thick | Lamina thick | palisade thick | Spongy thick | Dimensions of the main vascular bundle of mid vein | | Xylem tissue thick | Phloem tissue thick |
| | | | | | length | width | | |
| Control (100% FC ) | 930 | 165 | 68 | 82 | 385 | 405 | 177 | 135 |
| Control (50% FC ) | 905 | 175 | 70 | 90 | 265 | 320 | 160 | 110 |
| Nanocurcumin (3 mM) | 945 | 184 | 74 | 105 | 445 | 485 | 195 | 150 |
| Nanoglycyrrhizic acid (1 mM) | 1125 | 160 | 65 | 75 | 540 | 770 | 210 | 155 |

**Table (3): Effect of Nanocurcumin and Nanoglycyrrhizic acid under drought stress on anatomical characters of soybean stem.**

| | Anatomical characters of leaves | | | | | |
|---|---|---|---|---|---|---|
| Treatments | Main stem diameter | Cortex thick | Number of vascular bundles | phloem tissue thick | Xylem tissue thick | Parenchymatous pith thick |
| Control (100% FC ) | 3020 | 170 | 12 | 150 | 320 | 1555 |
| Control (50% FC ) | 2190 | 195 | 15 | 130 | 255 | 1330 |
| Nanocurcumin (3 mM) | 3177 | 165 | 18 | 215 | 590 | 1620 |
| Nanoglycyrrhizic acid (1 mM) | 3287 | 220 | 22 | 240 | 650 | 1685 |

## Growth characters

### Effect of drought

Results in Table (1) revealed that drought stress (50% FC) caused significant decreases for studied morphological characters compared to normal irrigation (100% FC). Plant height at 50% FC decreased significantly by 10.1% below plants irrigated with 100% FC. As to branches and leaves number recorded decrease in plants with 50% FC by 22.2 and 19.4% compared to control. The same trend was observed with fresh and dry weight of shoot, plants irrigated with 100% FC recorded the highest values, being 31.0 and 18.6% more than plants irrigated with 50% FC for plant fresh and dry weight, respectively.

### Effect of nanocurcumin

Data recorded in Table (4) indicated that all studied growth parameters of soybean; plant height, number of branches and leaves /plant, fresh and dry weights/plant were significantly affected by foliar spray with curcumin under water deficit in both seasons compared with untreated plants. Nanocurcumin at 6mM was the most effective treatment in increasing growth parameters in both seasons.

The maximum plant height of 88.27 cm was achieved at concentration of 6 mM nanocurcumin, being 71.3% more than control (50%FC). Concerning the number of branches, it is increased significantly with increasing the nanocurcumin concentration. Branches number were 10 at 3 mM, being 42.8% more than control, while at 6 mM recorded 11 being 57.1% over control. On the other hand, leaves number at 6 mM exhibited 69.5 comparing to 29.0 (control), being 139.6% more than control. As to the effect on fresh weight /plant, there was a significant increase in fresh weight with increasing the concentration. Data proved that plant recorded 140.1 and 143.0 g, being 140.0 and 145.3% more in comparison with the control for 3 and 6 mM, respectively. Regarding the effect on dry weight /plant, it is clear from Table (4) that, spraying plant with 6 mM recorded 63.4 g compared to 19.3 g for control, being 228.5% over control.

### Effect of nanoglycyrrhizic acid

As shown in Table (4) two concentrations with gnanolycyrrhizic acid were significantly increased all investigated morphological characters in both studied seasons compared with control. The most increasing of vegetation growth was observed when plants treated with 3 mM of nanoglycyrrhizic acid in both seasons. The highest concentration (3 mM) produced the tallest plants (88.5cm), being 71.8% more than control. Table (1) showed that plants treated with 1 mM recorded 9 branches, being 28.6% more than control whereas; at concentration 3 mM recorded 12 branches, being 71.4% over control. On the other hand, leaves number exhibited the highest number at 3 mM being 148.3% over control. The maximum fresh weight of plant 147.17g was obtained at 3 mM, being 155.7% more than control. The same trend was obtained with plant dry weight, at 1 mM exhibited 41.8g, being 116.6% more than control Table (4), while at 1 ml/l recorded the highest values 69.1 g, being 258.0% more than control.

**Table (4): Effect of irrigation, nutrition applications and its interaction on growth parameters of soybean.**

| Treatments | | Plant height (cm) | Number of branches / plant | Number of leaves/ plant | Shoot F.W\g | Shoot D.W\g |
|---|---|---|---|---|---|---|
| Irrigation (A) | Nutrition applications (B) | | | | | |
| Normal (100%FC) | Control | 56.43 | 8.67 | 36.00 | 84.50 | 23.37 |
| | Nanocurcumin (3 mM) | 79.93 | 10.00 | 55.00 | 134.33 | 53.33 |
| | nanocurcumin (6 mM) | 81.43 | 9.67 | 59.00 | 140.00 | 56.50 |
| | Nanoglycyrrhizic acid (1 mM) | 74.47 | 8.67 | 47.50 | 123.96 | 44.87 |
| | Nanoglycyrrhizic acid (3 mM) | 83.07 | 10.67 | 63.00 | 145.33 | 60.00 |
| Mean | | **75.07** | **9.53** | **52.10** | **125.48** | **47.61** |
| Drought (50%FC) | Control | 51.20 | 6.67 | 29.00 | 58.30 | 19.83 |
| | Nanocurcumin (3 mM) | 85.93 | 10.00 | 59.00 | 141.47 | 58.27 |
| | nanocurcumin (6 mM) | 88.27 | 10.67 | 69.50 | 143.83 | 63.47 |
| | Nanoglycyrrhizic acid (1 mM) | 79.37 | 8.66 | 53.50 | 102.53 | 42.00 |
| | Nanoglycyrrhizic acid (3 mM) | 88.50 | 12.00 | 72.00 | 147.17 | 69.70 |
| Mean | | **78.65** | **9.60** | **56.60** | **118.66** | **50.65** |
| General mean of nutrition applications (B) | Control | **53.82** | **7.67** | **32.50** | **71.40** | **21.60** |
| | Nanocurcumin (3 mM) | **82.93** | **10.00** | **57.00** | **137.90** | **55.80** |
| | Nanocurcumin (6 mM) | **84.85** | **10.17** | **64.25** | **141.92** | **59.98** |
| | Nanoglycyrrhizic acid (1 mM) | **76.92** | **8.67** | **50.50** | **113.25** | **43.43** |
| | Nanoglycyrrhizic acid (3 mM) | **85.78** | **11.33** | **67.50** | **146.25** | **64.85** |
| F test for | A | * | N.S | ** | * | N.S |
| LSD at 0.05 of | B | 4.24 | 0.78 | 1.122 | 2.59 | 1.74 |
| | AxB | 3.45 | 1.11 | 1.73 | 3.67 | 2.46 |

## Yield and its contents

Table (5) revealed that water defect (50%FC) decreased the number of pods/plant by 50.7% in comparison with the control. On the other hand, weight of 100 seeds decreased below control by 2.2%. Finally seed and total yield of plant decreased as a result of water defect by 47.3 and 48.8 % below control. Relative to the control, plants showed gradually increasing in pods number as curcumin concentration increased. Which increased by 163.0 and 175% at 0.5 and 1.0 ml/l, respectively. Weight of 100 seeds increased over control by 9.6 and 15.5 % at the same previous concentrations, respectively. Spray nanocurcumin at 3mM increased seed and total yield by 26.6 and 29.3% over control. As to the effect of nanoglycyrrhizic acid, it is clear that the number of pods/plant exhibited the highest at 3 mM in comparison with control by 210 %. Spraying nanoglycyrrhizic acid at 3 mM recorded the highest weight of 100 seeds by 20.7 % over control. Seed and total yield increased by 42.7 and 43.9 % over control as a result of spray nanoglycyrrhizic acid at 3 mM.

**Table (5): Effect of irrigation, nutrition applications and its interaction on the yield of soybean.**

| Treatments | | Number of pods/plant | 100 seed weight g. | Seed yield /plant (g.) | Plant yield t/h |
|---|---|---|---|---|---|
| **Irrigation (A)** | **Nutrition applications (B)** | | | | |
| **Normal (100%FC)** | **Control** | 138.00 | 13.87 | 41.93 | 0.41 |
| | Nanocurcumin (3 mM) | 145.00 | 13.67 | 49.00 | 0.44 |
| | nanocurcumin (6 mM) | 170.00 | 15.13 | 51.60 | 0.46 |
| | Nanoglycyrrhizic acid (1 mM) | 139.00 | 13.60 | 41.97 | 0.39 |
| | Nanoglycyrrhizic acid (3 mM) | 178.00 | 15.47 | 57.00 | 0.53 |
| **Mean** | | **154.00** | **14.35** | **48.10** | **0.45** |
| **Drought (50%FC)** | **Control** | 68.00 | 13.50 | 22.00 | 0.21 |
| | Nanocurcumin (3 mM) | 179.00 | 14.93 | 49.87 | 0.50 |
| | nanocurcumin (6 mM) | 187.00 | 15.80 | 51.80 | 0.52 |
| | Nanoglycyrrhizic acid (1 mM) | 161.00 | 13.90 | 44.86 | 0.45 |
| | Nanoglycyrrhizic acid (3 mM) | 211.00 | 15.79 | 58.93 | 0.59 |
| **Mean** | | **161.20** | **14.78** | **45.49** | **0.45** |
| **General mean of nutrition applications (B)** | **Control** | **103.00** | **13.68** | **31.46** | **0.31** |
| | Nanocurcumin (3 mM) | **162.00** | **14.30** | **49.43** | **0.47** |
| | Nanocurcumin (6 mM) | **178.50** | **15.47** | **51.70** | **0.49** |
| | Nanoglycyrrhizic acid (1 mM) | **150.00** | **13.75** | **43.42** | **0.42** |
| | Nanoglycyrrhizic acid (3 mM) | **194.50** | **15.62** | **57.97** | **0.56** |
| F test for | **A** | ** | N.S | ** | N.S |
| **LSD at 0.05 of** | **B** | 1.34 | 0.73 | 0.51 | 0.013 |
| | **AxB** | 1.89 | N.S | 0.73 | 0.018 |

## Mineral elements content in leaves

Data presented in Table (6) illustrated the effect of the foliar application with two concentrations of nanocurcumin or nanoglycyrrhizic acid on percentage of nitrogen, phosphor, potassium and total chlorophyll in dry leaves under drought stress and natural infestation of spider mite, *T. urticae*. It is obvious from leaves analysis that, there are differences between the two studied materials where curcumin at 3 mM recorded an increase by 37.1, 31.0 and 26.8 for the percentage of N, P and K, respectively more than control, while spraying at 6 mM recorded an increase by 39.9, 44.8 and 31.4% respectively more than control for the previous elements. As to total chlorophyll percentage, treatment with 1.0 ml/L gave the highest increase percentage (71.7%) more than untreated plants (control). Foliar application of nanoglycyrrhizic acid at 3 mM significantly increased the percentage of N, P and K by 61.5, 65.5 and 39.4% respectively. Concerning the effect of nanoglycyrrhizic acid on percentage of total chlorophyll, spray with 1 mM recorded the highest percentage (85.1%) more than control (Table 6).

**Table (6): Effect of Nanocurcumin and Nanoglycyrrhizic acid under drought stress on mineral elements content in soybean leaves of. Each value is average of both seasons.**

| Treatments | | N% | P% | K% | Total chlorophyll | Crude protein |
|---|---|---|---|---|---|---|
| Control (100% FC ) | | 2.95 | 0.19 | 1.89 | 3.31 | 18.43 |
| Control (50% FC ) | | 2.86 | 0.29 | 1.75 | 2.69 | 17.87 |
| Nanocurcumin | (3 mM) | 3.92 | 0.38 | 2.22 | 4.11 | 24.50 |
| | (6 mM) | 4.00 | 0.42 | 2.30 | 4.62 | 25.00 |
| Nanoglycyrrhizic acid | (1 mM) | 3.81 | 0.37 | 1.93 | 4.09 | 2381 |
| | (3 mM) | 4.62 | 0.48 | 2.44 | 4.98 | 28.87 |

## Chemical studies

### Determination of amino acids in seeds

Analysis of amino acids in soybean seeds detected ten types (Table 7). These types were divided into essential amino acids i.e., Methionine, Tryptophan, Leucine and Lysine and nonessential amino acids, i.e., Glutamate, Glycine, Alanine, Arginine, Tyrosine and Aspartate. Seeds of soybean contain high concentration of Glutamate, moderate concentration of Aspartate, Leucine, Arginine, Lysine, Glycine, Alanine and Tyrosine, and low concentration of Methionine and Tryptophan. Regarding the effect of treatment with two concentrations of nanocurcumin or nanoglycyrrhizic acid on the amino acids content of soybean seeds, the obtained results showed that foliar spraying nanoglycyrrhizic acid at 3 mM caused a marked increase in the concentration of all essential and nonessential amino acids comparing with the control plants. The increases percentage in the amino acids content recorded 4.5% in Glutamate, 21.4% in Glycine, 37.5% in Methionine, 18.5% in Alanine, 12.4% in Arginine, 28.6% in Tyrosine, 24.6% in Tryptophan, 13.6% in Leucine, 7.8% in Lysine and 4.7% in Aspartate compared with untreated plants.

**Table (7): Effect of nanourcumin and nanoglycyrrhizic acid under drought stress on distribution of amino acids in soybean seeds. Each value is average of both seasons**

| Treatments | | Glutamate | Glycine | Methionine | Alanine | Arginine | Tyrosine | Tryptophan | Leucine | Lysine | Aspartate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control (100% FC ) | | 79.6 | 19.5 | 5.7 | 16.8 | 27.6 | 13.4 | 5.6 | 32.2 | 23.5 | 40.8 |
| Control (50% FC ) | | 77.8 | 18.2 | 4.8 | 16.2 | 25.8 | 11.9 | 5.7 | 30.8 | 23.1 | 40.2 |
| Nanocurcumin | 3mM | 80.7 | 20.6 | 6.4 | 18.0 | 28.5 | 14.8 | 6.4 | 33.4 | 24.5 | 41.6 |
| | 6mM | 80.8 | 21.5 | 6.8 | 16.5 | 26.7 | 15.5 | 6.5 | 32.4 | 22.5 | 40.6 |
| Nanoglycyrrhizic acid | 1mM | 80.5 | 20.3 | 6.0 | 17.1 | 28.4 | 14.5 | 6.1 | 33.0 | 24.1 | 41.5 |
| | 3mM | 81.3 | 22.1 | 6.6 | 19.2 | 29.0 | 15.3 | 7.1 | 35.0 | 24.9 | 42.1 |

## Leaf anatomy

It is recognized from Table (2) and Figure (7) that drought stress (50% FC) caused significant decreases in all anatomical characters except lamina, palisade and spongy tissues thickness. Whereas mid vein, xylem and spongy tissues decreased by 2.7, 9.6 and 18.5% below control. On the other hand, there is an increase in lamina thickness by 6.1% due to the increase in palisade and spongy tissues by 2.9 and 12.1%, respectively. Exogenous application with 3 mM nanocurcumin increased the thickness of midvein and lamina by 4.4 and 5.1% respectively, more than the control (50% FC). It is cleared that the increase in lamina thickness was accompanied by increments in thickness of palisade and spongy tissues by 5.7 and 16.7 % at 3mM over the control, respectively. Likewise, the main vascular bundle of the midvein was increased in size as a result of spray with nanocurcumin at 3mM. The increment was mainly due to the increase in xylem by 21.9 % and phloem by 36.4% more than the control.

Concerning the effect of nanoglycyrrhizic acid on leaf structure, it was observed in the same table that, the thickness of midvein, xylem and phloem tissues increased markedly at 1 mM over the untreated plants by 24.3, 31.2 and 40.9 %, respectively. As well as the thickness of lamina, palisade and spongy tissues decreased by 8.6, 7.1 and 16.7 %, respectively at 1 mM as compared to non-treated plants.

### Stem anatomy

Table (3) and Figure (8) revealed that water deficit (50% FC) caused significant decreases in all anatomical characters except cortex thickness. Whereas main stem diameter decreased by 27.5% below control. Number of vascular bundles was increase by 25% compared with control. While thickness of phloem and xylem tissues and parenchymatous pith decreased by 13.3, 20.3 and 14.5% respectively, compared with control. On the other hand, there is an increase in cortex thickness by 14.7%. Data presented in Table (3) and Figure (8) indicated that foliar spray with curcumin increased the diameter of the main stem by 45.1% at 6 mM more than that of the control. Number of vascular bundles was increase by 20.0 % more than control. The increase thickness of phloem and xylem tissues and parenchymatous pith at 1.0 ml/l were, 65.4, 131.4 and 21.8 % more than those of the control, respectively. While thickness of cortex decreased by 15.4% less than control. Moreover, the thickness of main stem increased with application of nanoglycyrrhizic acid at 3 mM over control by 50.1%. Number of vascular bundles was increase at 1 mM by 46.7 %. On the other hand, the thickness of cortex, phloem, xylem tissues and parenchymatous pith was markedly increased under the same conditions by 12.8, 84.6, 154.9 and 26.7% respectively.

## Discussion

Soybean (*Glycine max* (L.) Merrill) is one of the most widely grown and used oilseeds. Usage of soybean vary from human to animal foods, industrial products, ingredients, and precursor materials. A key step to utilizing soybeans is to separate oil from protein and fiber. Both biotic and abiotic stresses can cause variability of the biomass production and secondary metabolites contents. Therefore, improving biomass production and induction of specific secondary metabolite pathways to boost the benefits of these crops became the main target for many companies and farmers. Safe And Sound materials for human being and environment should be employed to induce specific secondary metabolites and enhance plant resistance against different stimuli.

Nanotechnology has been considered as one of the most important technologies for the modern Era. Nanotechnology has been applied in agriculture with great impact in many agricultural disciplines. For instance, natural nanomaterials can enhance plant growth, secondary metabolites, active compounds, resistance to various stimuli, crop yields and distinct physiological pathways. Our current research studies proofed that the application of nanoglycyrrhizic acid and nanocurcumin on the Soybean (*Glycine max)*, enhanced the photosynthesis rate by increasing Leaf area root length, water and nutrient materials absorption, which impacted in the percentage of chlorophyll, plant height, number of branches, fresh and dry weight, oil percentage and contents and plant resistance against both biotic and abiotic stresses (Tables 2, 3) (Figs. 6a, 6b).

Exogenous application of nanoglycyrrhizic acid and nanocurcumin on the Soybean (*Glycine max*) may induce certain pathways which impact greatly in the plant growth, physiological processes, secondary metabolites, different pathways and active compounds production. Reactive Oxygen species (ROS) genes are the first defense system pathway which usually upregulated within seconds to minutes in response to biotic and abiotic stress, furthermore, could act as protectants from different stresses and signals translocator and activator (Jiang et al., 2007). Also, induced many pathways which affect positively on the plant. Induction of Reactive Oxygen Species (ROS) within seconds to minutes after treatment with of nanoglycyrrhizic acid and nanocurcumin resulted in full activation of Mitogen-activated protein kinases (MAPKs) genes.

Nanoglycyrrhizic acid and nanocurcumin exogenous application on plants induce MAPKs which regulate many important cellular processes including cell division, different developmental processes regulated by hormones, stress responses, metabolism, and biologically active compounds (Jonak et al., 2002). External application of nanoglycyrrhizic acid and nanocurcumin at certain concentrations induce Oxidative signal Inducible1 (Oxi1) (serine/threonine kinase), a member of AGC family of protein kinase. Protein kinase linking ROS accumulation to plant response and resistance to variable stimuli and oxidative burst-mediated signaling in plant roots (Rentel et al., 2004; Shoala et al., 2018). Furthermore, MAPKs genes are very important key player in calcium signaling pathways which in turn control stomata opening and closure, callose deposition and seeds germination (Peterson et al., 2009).

Exogenous application of nanoglycyrrhizic acid and nanocurcumin showed that the role MAPKs cascades also can control leaf extension and root length in response to different stimuli. Our results revealed that

nanoglycyrrhizic acid and nanocurcumin stimulates MAPKs cascades which enhanced plant growth which resulted from increasing the photosynthesis pathways growth hormones (Tables 2, 3). Also, stimulate of MAPKs cascades led to an increase in trichrome glandular density which affected positively in the phytohormone levels. Stimulation of the phytohoromone levels induces the plastidic MEP (Methyl Erythritol Phosphate) and enzymes of cytosolic MVA (mevalonate) pathway. Both MEP and MVA pathways stimulate IPP (Isopentenyl Pyrophosphate) and DMAPP (Dimethylallyl Pyrophosphate) are very important in the induction of Terpene synthesis pathway ended by production of terpenoids like Sabinene, β - phyllandrene and other essential oils which affect positively on the quality and quantity of certain important essential oils and improve the resistance level against different stimuli (Fig. 9). Nutrient materials and water uptake enhanced because of increasing root length and leaf area. Nitrogen uptake, which is considered as an important fundamental constituent of amino acids and enzymes, its sufficiency would positively affect terpenoid biosynthesis. Nitrogen sustains terpenoid productions by improving electron transport rate and photosynthesis process (Ormeno and Fernandez, 2012).

Mineral elements content in leaves also has accordance with result of Panjtan-Doost (1999) about the positive impact of iron on increasing phosphorous content in peanut kernels. The storage form of phosphorous in seeds is phytic acid (phytin) and it seems that increasing of it, have major efficacy in seeds germination rate. Liu *et al*. 2005 showed that iron nano-carbonate had positive effect on protein content in peanut. Also the positive effect of iron on protein content have been reported by Hemantarajan and Trivedi 1997, Rahman 1992 in soybean. Krishnaraj et al. (2012) and Salama *et al.* (2012) stated that foliar spraying with FeO or MgO NPs significantly increased protein (%) of the yielded seeds in *Phaseolus vulgaris* and *Bacopa monnieri*, respectively. Hatami, and Ghorbanpour, (2013) and. Lu et al. (2002) showed that nanoparticles play an important role in improving photosynthetic quantum efficiency and chlorophyll content and increased water and fertilizer use efficiency. Siva and Benita. (2016) found that ginger plants exposed to iron oxide nanoparticles respond at molecular level with slight increase in the amount of protein and there is expression of some bands due to increase in the total protein amount which was not present in control. Roghayyeh

(2018) stated that maximum quantity of chlorophyll-a (0.190 Mg $g^{-1}$) and phosphorus was achieved in 0.75 g $L^{-1}$ of nano-iron oxide that has significantly difference with other levels of nano-$Fe_2O_3$ and control in soybean seeds.

The present results were similar to those obtained by El-Feky et al. (2013) on basil who found that application of nano Fe3O4 improved anatomical structure of leaves, and Abdel Wahab et al. (2017) on *Carum carvi* who declared that, application of nano-zeolite enhanced anatomical structure compared to control (NPK). Mahmoud and Swaefy 2020 mentioned that differences involved 20% increase in lamina thickness of plants treated with (T7) due to the increase in thickness of upper epidermis (33.3%), palisade tissue (16.7%), and spongy tissue (17.8%). There was also an increase in the dimensions of the midrib as the increase percentages were 42.9% in length and 87.3% in width, which refers to the increase in the dimensions of the midrib vascular bundle, as it reached 50% in length and 200% in width, along with the thickness of the collenchyma above the vascular bundle (40%) and below the vascular bundle (33.3%) as well as the thickness of the parenchyma above the vascular bundle (106.8%) and below the vascular bundle (52.5%).The increase in the dimensions of the midrib vascular bundle is due to the increase in the thickness of the phloem tissue by 80% and xylem tissue by 71.4%.

Raliya et al. 2015 showed a better growth of *Solanum lycopersicum*, increase in fruit yield due to TiO2 NPs foliar treatment. Razzaq et al. 2016 induced the highly favorable effects of Ag NPs on number of grains/spike, 100-grain weight and grain yield per pot in wheat.

Histological leaf characteristics vary from one treatment to another, which may affect the population levels of herbivores. The lowest density of phytophagous mites occurred on leaves which had a thicker palisade mesophyll. Some varieties with thicker cuticle and epidermis were more resistant to spider mite. Besides that, the upper leaf surface was less preferred by mites, because its cuticle and epidermis are thicker than that of the lower surface (Ali et al., 2015; Alakhdar, 2016). Mites exclusively feed on cells within the mesophyll-parenchyma, most frequently; the disturbed cells were adjacent to the epidermal layer of the leaf surface that mites fed from. As leaf thickness ranges between100 and 150µm, mites stylet can completely transverse a leaf, allowing it to reach either the palisade or spongy mesophyll regardless of the leaf surface mites are on. The basis for this preference is currently not known (Bensoussan et al., 2016).

The induced responses of the host plant that reduces spider mites survival, reproductive output, or preference for a plant is a biochemical mechanism of protection against pests that are wide-ranging, highly dynamic, and are mediated both by direct and indirect defenses. Moreover, foliar application of nanocurcumin and nanoglycyrrhizic leads to an increase of nitrogen, potassium, phosphorus content and enhances the photosynthetic pigments, capacity, and subsequently which is unfavorable for *T. urticae* and directly caused an observed reduction in its populations (Mondal et al., 2017; **Tien** et al., **2010).**

# Conclusion

Both nanocurcumin and nanoglycyrrhizic acid may act as nano fertilizer sources alleviate the harmful effect of water deficiency for plant growth and its yield compared with untreated plants. Glycyrrhizic acid at 3 mM had the highest values for morphological, yield and anatomical characters. Spraying plants with glycyrrhizic at 50% of field capacity increased the percentages of, nitrogen, phosphor and potassium, in seeds, in addition to total chlorophyll. Mean number of mites reduced significantly in response to Nanocurcumin 3mM in comparison with the other treatments both normal and drought conditions. Application of glycyrrhizic at 3 mM showed high concentration of glutamate; moderate of aspartate, leucine, arginine, Lysine, glycine and tyrosine and low of tryptophan and methionine.

## Declarations

Ethical Approval and Consent to Participate: Not Applicable (My research did not deal with animal or human experiments)

Consent to Publish and Authors Contributions

I am aware of the contents and consent to the use of my name as an author of a manuscript entitled: Influence of spraying Nano-curcumin and Nano- glycyrrhizic acid on resistance enhancement and some growth parameters of soybean (Glycine max) in response to *Tetranychus urticae* infestation and drought stresses.

All authors have participated in Idea, hypothesis, Experimental design, writing up, interpretation and review.

The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

| Author's name | Affiliation | Contribution | Percentage |
|---|---|---|---|
| Azza M. Salama | Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt. | Idea, hypothesis, Experimental design, writing up, interpretation and review. | 33.3% |
| Hala H. Alakhdar | Cotton and Crops Acarology Department, Plant Protection Research Institute, Agricultural Research Center, Egypt. | Idea, hypothesis, Experimental design, writing up, interpretation and review. | 33.3% |
| Tahsin Shoala | Environmental Biotechnology Department, College of Biotechnology, Misr University for Science and Technology, Egypt. | Idea, hypothesis, Experimental design, writing up, interpretation and review. | 33.3% |

Our research is not involving human participants or animals. This investigation was occurred to study the response of soybean plant to the application of curcumin nanoparticles and Nano-glycyrrhizic acid under drought condition and its relation to the population of the two-spotted spider mite Tetranychus urticae. Our main goal is using safe and environmentally friendly treatments without harming human being and the environment.

I understand that the staff of the Journal will make every effort to keep such information confidential during the editorial review process. I also understand that if the manuscript is accepted, you will discuss with me the manner in which such information is to be communicated to the reader. I hereby grant permission for any such information, to be included with publication of the manuscript in the Environmental Science and Pollution Research journal.

Funding: Not Applicable

Competing Interests: "Authors have no competing interests to declare"

Availability of data and materials:

The data that support the findings of this study are available on request from the corresponding author "Tahsin Shoala"

## References

**Abd El-Mohsen RA, El-Bassiouny, MSH, Bakry, AB, Abdallah, MSM, El-Enany, AMM. 2020.** Growth, Yield and Biochemical Changes of Soybean Plant in Response to Iron and Magnesium Oxide Nanoparticles. Pak. J. Biol. Sci., 23 (3): 406-417.

**Abdel-Rahman, F.A, Rashid, I., Shoala, T. 2020.** Nanoactivities of natural nanomaterials rosmarinic acid, glycyrrhizic acid and glycyrrhizic acid ammonium salt against tomato phytopathogenic fungi *Alternaria alternata* and *Penicillium digitatum*. Journal of Plant Protection Research, 60, 150–160.

**Abdel Wahab, MM, El-attar, AB, Mahmoud, AA. 2017.** Economic evaluation of nano and organic fertilisers as an alternative source to chemical fertilisers on *Carum carvi* plant yield and components. Agric. (Poľnohospodárstvo), 63 (1): 33–49.

**Alakhder, HH, Ghareeb, EZ, Rabie EM. 2015.** Evaluation Some Genotypes of Soybeans Yield Under Pest Infestation. *Internatial Journal of Scientific Resarch Agricultural Science.,* 2: 007-017. DOI:10.13140/RG.2.2.15939.14888

**Alakhder, HH. 2016.** Newly Selected Soybean Genotypes and Their Resistance Against The Two-Spotted Spider mite *Tetranychus urticae* Koch. ACARINES, 10: 65-70.

**Alakhder, HH. 2020.** Efficacy of Chitosan Nano-particles Against Two Tetranychid mites and Two Associated Predaceous Mites (Acari: Tetranychidae: Phytoseiidae). *Egyptian Scientific Journal of Pesticides*,6 (1); 8 – 13. DOI:10.13140/RG.2.2.20972.31363/3.

**Ali, SF, Afifi, AM, El-Saiedy, EMA, Ahmed, MM. 2015.** Effect of hytochemical Components, Morphological and Histological Leaf Structure of Five Tomato Hybrids on *Tetranychus urticae* Koch Infestation. ACARINES, 9:23-30. https://www.researchgate.net/publication/289128451

**Arimura GI, Matsui K, Takabayashi J. 2009**. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol., 50:911-923.

**Bensoussan, N, Santamaria, ME, Zhurov1,V, Diaz, I, Grbi ´ c1, M, Grbi ´ cl, Z. 2016**. Plant-Herbivore Interaction: Dissection of the Cellular Pattern of Tetranychus urticae Feeding on the Host Plant. Frontiers in Plant Science. 7: 1-13. https://doi.org/10.3389/fpls.2016.01105.

**Dhoke, SK., P. Mahajan, RK, Khanna, A. 2013.** Effect of nanoparticles suspension on the growth of mung (*Vigna radiata*) seedlings by foliar spray method. Nanotechnol. Dev., Vol. 3, No. 1.

**El-Feky, SA, Mohammed, MA, Khater, MS, Osman, YA, Elsherbini, E. 2013.** Effect of magnetite nano-fertilizer on growth and yield of *Ocimum basilicum* L. Int. J. Indigenous Med. Plants, 46 (3): 1286-1293.

**Georgieva, N, Nikolova, I. 2019.** Menu Script Field: Response of Soybean to Water Deficit and Spider Mites during Seed Filling Stage. Sumerianz Journal of Agriculture and Veterinary. 2 :( 7) 40-48. panjtan-doost M. 1999. Effect of iron on the quantity and quality of peanuts (*Arachis Hypogaea* L.) in Gilan province. Ms.C thesis. Tarbiat Modares University. Iran.

**Gillman, JH, Rieger, MW, Dirr MA, Braman, SK. 1999.** Drought stress increases densities but not populations of two-spotted spider mite on *Buddleia davidii* 'pink Delight'. HortScience*, 34*, 280-282.

**Haack, RA, Slansky, FJr. 1987.** Nutritional ecology of wood-feeding Coleoptera, Lepidoptera, and Hymenoptera. In Slansky, F.Jr. and Rodriguez, J.G. (Eds.). The Nutritional Ecology of Insects, Mites, and Spiders, (pp. 449-486). New York, NY: John Wiley & Sons.

**Hammam KA, Shoala T. 2020.** Influence of spraying Nano-curcumin and Nano-rosemarinic acid on growth, fresh herb yield, chemicals composition and postharvest criteria of French basil (*Ocimum basilicum* L. var. Grand Vert) plants, Journal of Agricultural and Rural Research, 5(1): 1-22.

**Hanley, ME, Lamont, BB, Fairbanks, MM, Rafferty, CM. 2007**. Plant structural traits and their role in antiherbivore defense. Perspec. Plant Ecol Evol Syst, 8:157-78.

**Hatami, M, Ghorbanpour, M. 2013.** Effect of nanosilver on physiological performance of pelargonium plants exposed to dark storage. J. Hortic. Res., 21: 15-20.

**Hemantarajan A, Trivedi Ak. 1997.** Growth and yield of soybean as influenced by sulphur and iron nutrition. Indian J Plant Phys 2: 304-306.

**IHIDMA. 2002.** Pharmacopoeia. Indian Herbal Indian Drug Manufacturers Association, Mumbai.

**Jiang PD, Zhu YG, Wang XL, Zhu W, Zhang XG, Xie HY, Wang XD. 2007.** Metabolism of reactive oxygen species in the cytoplasmic male-sterile cotton anther. Agric. Sci. Chin. 6(3): 275-280.

**Jonak C, Okrész L, Bögre L, Hirt H. 2002.** Complexity, cross talk and integration of plant MAP kinase signalling. Curr Opin Plant Biol. 5(5):415-24. doi: 10.1016/s1369-5266(02)00285-6. PMID: 12183180.

**Krishnaraj, CEG, Jagan, R, Ramachandran, SM, Abirami, NM, Kalaichelvan, PT. 2012.** Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. Process Biochem., 47: 651-658

**liu xm, zhang fr, feng zb, zhang shq, he xsh, wang r, wang. y. 2005.** Effects of nano-ferric oxide on the growth and nutrients absorption of peanut. Plant Nutr Ferti Sci 11: 14-18.

**Lu, C, C. Zhang, J, Wen, G, Wu, Tao M. 2002.** Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Sci., 21: 168-171.

**Mohamed AM, Swaefy MH. 2020.** comparison between effect of commercial and nano npk in presence of nano zeolite on sage plant yield and components under drought stress. Zagazig J. Agric. Res., Vol. 47 No. (2).

**Mondal A, Uma Shankar U, Abrol DP, Singh I, Norboo T. 2017.** Evaluation of pest management strategies against sucking insect-pests for the safety of beneficial insects in vegetable French bean (*Phaseolus vulgaris* L.). Int J Curr Microbiol App Sci 6 (8):1441-1448. https://doi.org/10.20546/ijcmas.2017.608.174.

**Nikolova, I, Georgieva, N, Jordanka N. 2014.** Development and reproduction of spider mites *Tetranychus turkestani* (Acari: Tetranychidae) under water deficit condition in soybeans. Pestic. Phytomed. (Belgrade). 29(3): 187–195. DOI: 10.2298/PIF1403187N.

**Peterson, ER, Rayner, SG, Armstrong, S.J. 2009.** Researching the psychology of cognitive style and learning style: Is there really a future? Learning and Individual Differences, 19, 518-523.

**Poovizhiraja, B, Chinniah, C, Ravikumar, A. 2017.** Influence of Irrigation Schedule on the Biological Parameters of TSSM *T. urticae* Koch. on Okra, Eggplant and Tomato. Int.J.Curr.Microbiol.App.Sci. 6 (10): 1731-1738.

**Rahman MM. 1992.** Effect of micronutrient on the growth and yield of soybean plants. $4^{th}$-$5^{th}$ Bangladesh science conference, p. 122.

**Raliya, R, Nair, R, Chavalmane, S, Wang, WN, Biswas, P. 2015.** Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. Metallomics, 7: 1584-1594.

**Razzaq, A, Ammara, R, Jhanzab, HM, Mahmood, T, Hafeez, A, Hussain, S. 2016.** A novel nanomaterial to enhance growth and yield of wheat. J. Nanosci. Technol., 2: 55-58.

**Remadevi, R, Surendran, E, Kimura, T. 2007.** Turmeric in Traditional medicine. In Turmeric: the genus Curcuma, Ravindran, P. N.; Nirmal Babu, K.; Sivaraman, K., Eds. CRC Press: Boca Raton, London, New York, 409-436.

**Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Knight H, Peck SC, Grierson CS, Hirt H, et al. 2004.** Oxi1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis. Nature. 427: 858–861.

**Salama, HMH. 2012.** Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Int. Res. J. Biotechnol., 3: 190-197.

**Sasikumar, B. 2005.** Genetic resources of Curcuma: diversity, characterization and utilization. Plant Gen. Resour., 3, 230-251.

**Sheykhbaglou, R, Sedghi, M, Fathi-Achachlouie B. 2018.** The Effect of Ferrous Nano-oxide Particles on Physiological Traits and Nutritional Compounds of Soybean (*Glycine max* L.) Seed. An Acad Bras Cienc , 90 (1).

**Shoala, T. 2018.** Positive impacts of nanoparticles in plant resistance against different stimuli 267–279. In: "Nanobio- technology applications in plant protection" (K.A. Abd- -Elsalam, R. Prasad, eds). 1st ed. Springer International Publishing AG, part of Springer Nature.

**Shoala, T, Edwards, MG, Knight, MR, Gatehouse AMR. 2018.** OXI1 kinase plays a key role in resistance of Arabidopsis towards aphids (*Myzus persicae*). Transgenic Res 27: 355.

**Siva, GV, Benita, LFJ. 2016.** Iron oxide nanoparticles promotes agronomic traits of ginger (*Zingiber officinale* Rosc.). Int. J. Adv. Res. Biol. Sci., 3: 230-237.

**War, AR, Paulraj, MG, Ahmad, T, Buhroo, AA, Hussain, B, Ignacimuthu, S, Sharma, HC. 2012.** Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 7(10): 1306–1320.

# Figures

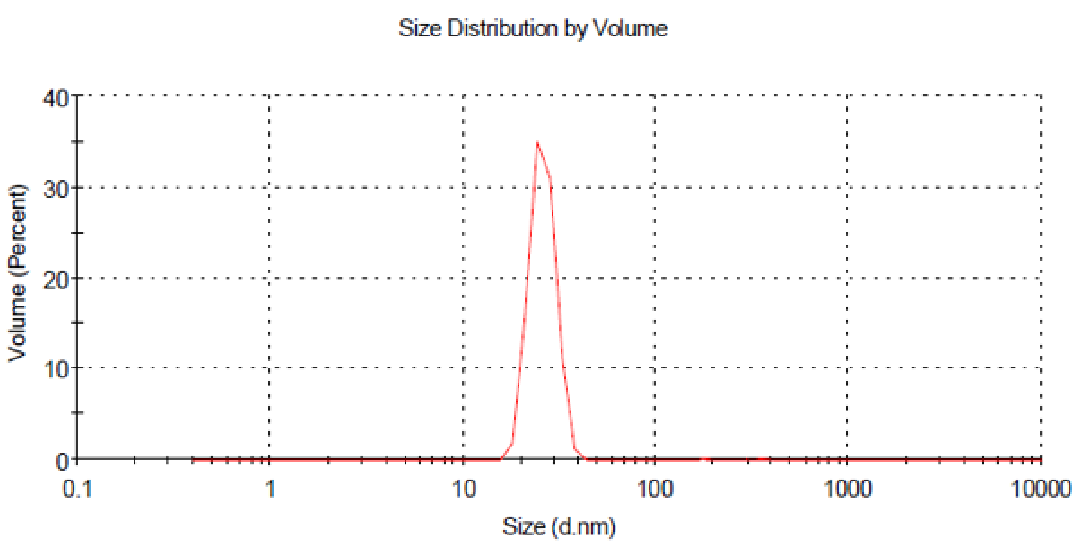



**Figure 1**

Z average size of curcumin nanoparticles.

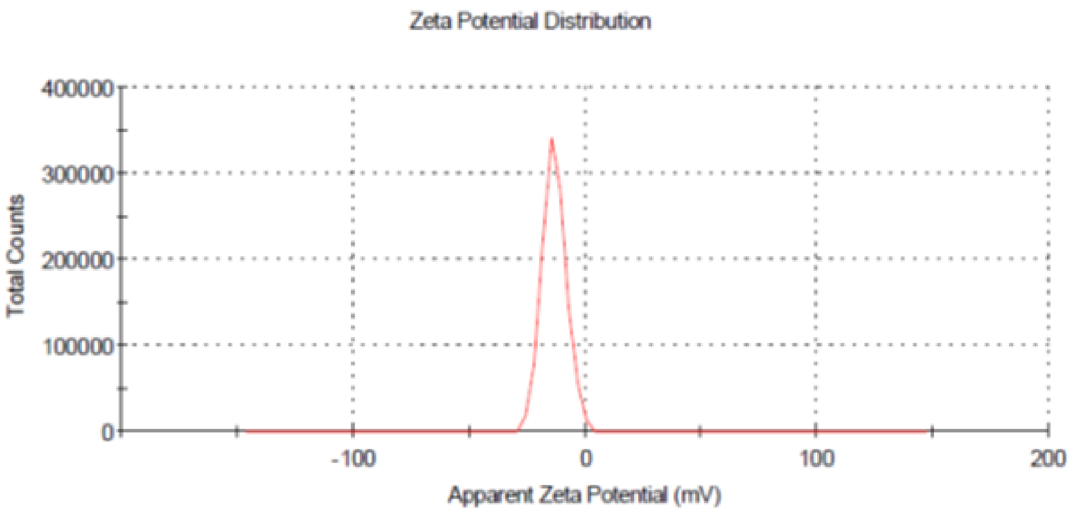



**Figure 2**

Zeta potential for curcumin nanoparticles.

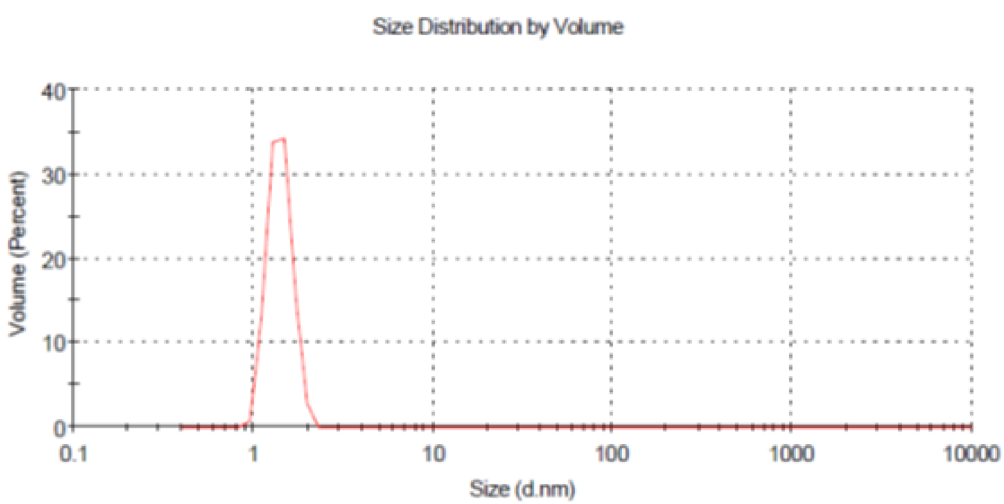
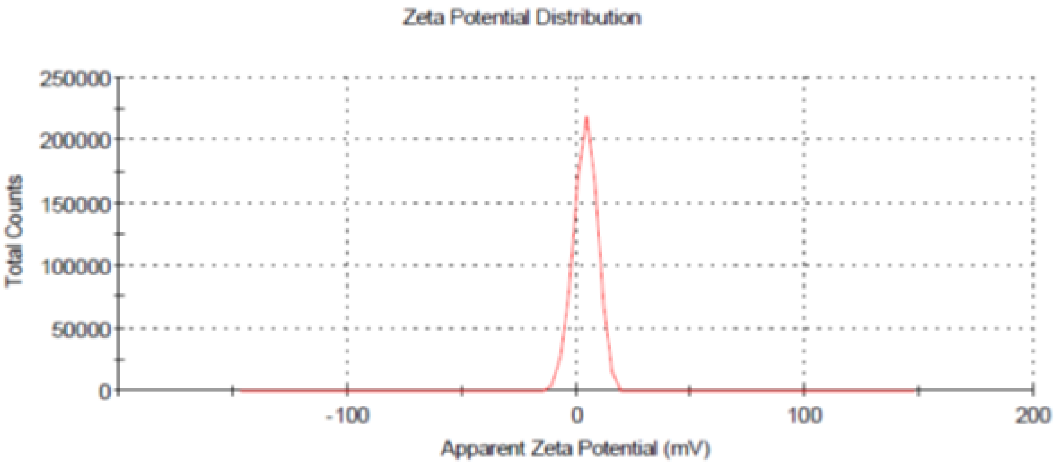


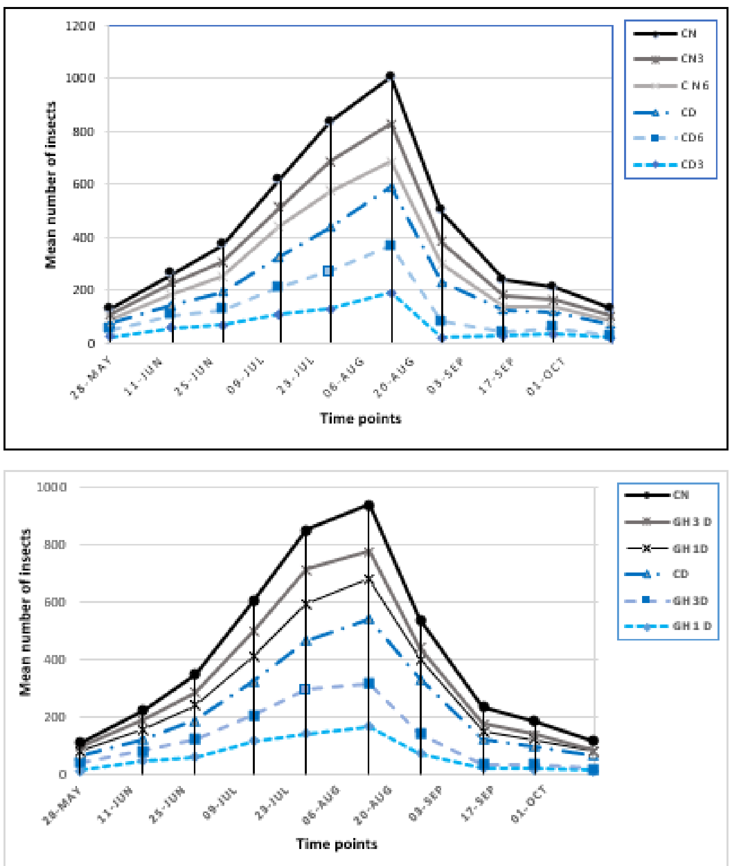

**Figure 3**

Z average size of Glycyrrhizic acid nanoparticles.



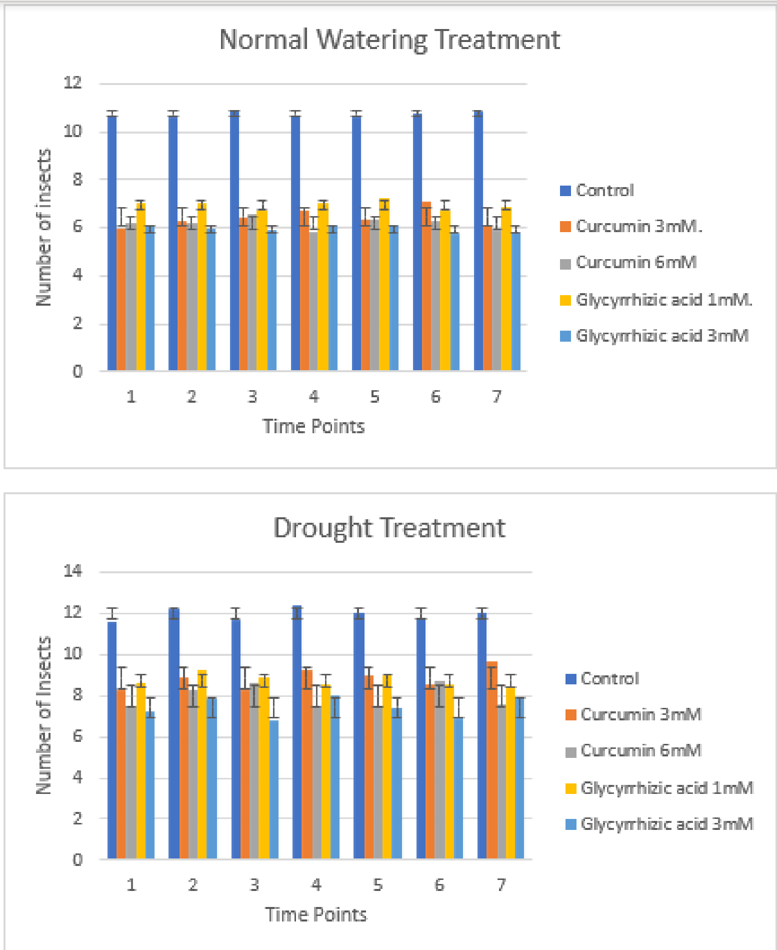

## Figure 4

Zeta potential for of Glycyrrhizic acid nanoparticles.




## Figure 5

a. Mean of seasonal abundance of mites infesting soybean plants under treatments by curcumin nanoparticles (CU-NPs) 3 and 6 mM during the successive growing seasons of 2018-2019. b. Mean of seasonal abundance of mites infesting soybean plants under treatments by Glycyrrhizic acid nanoparticles (GA-NPs) 1 and 3mM during the successive growing seasons of 2018-2019.




## Figure 6

a. Number of T. urticae at different time points in response to normal watering treatment. b. Number of T. urticae at different time points in response to drought treatment.

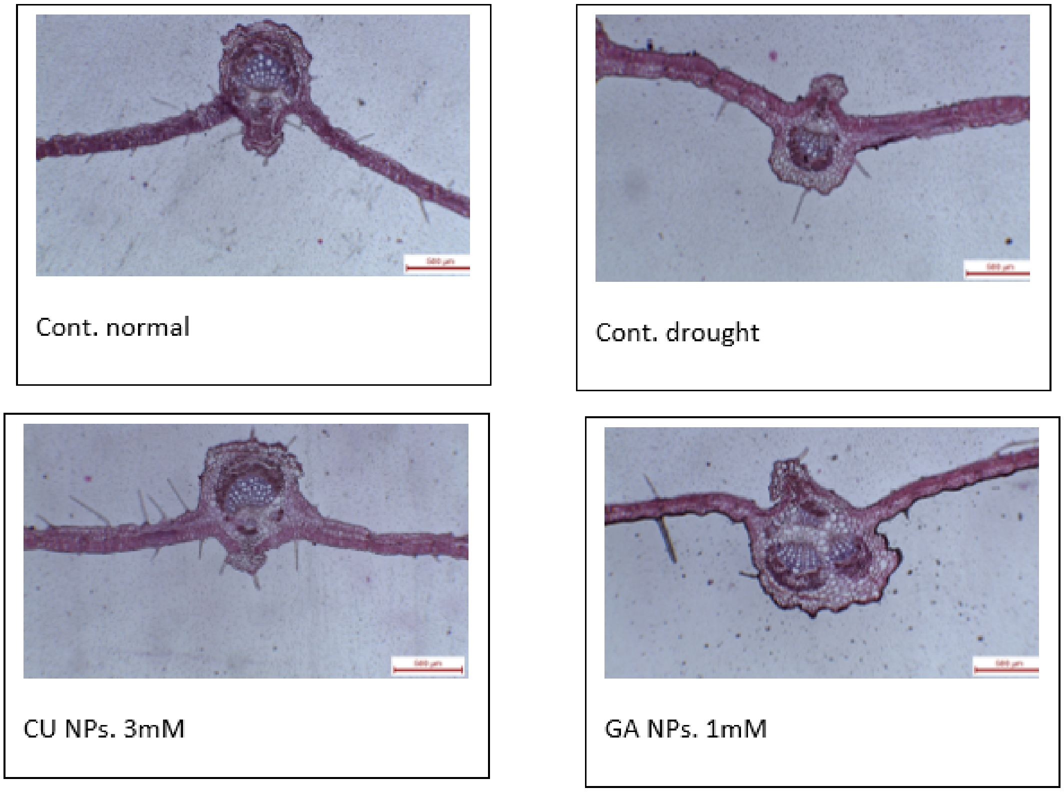
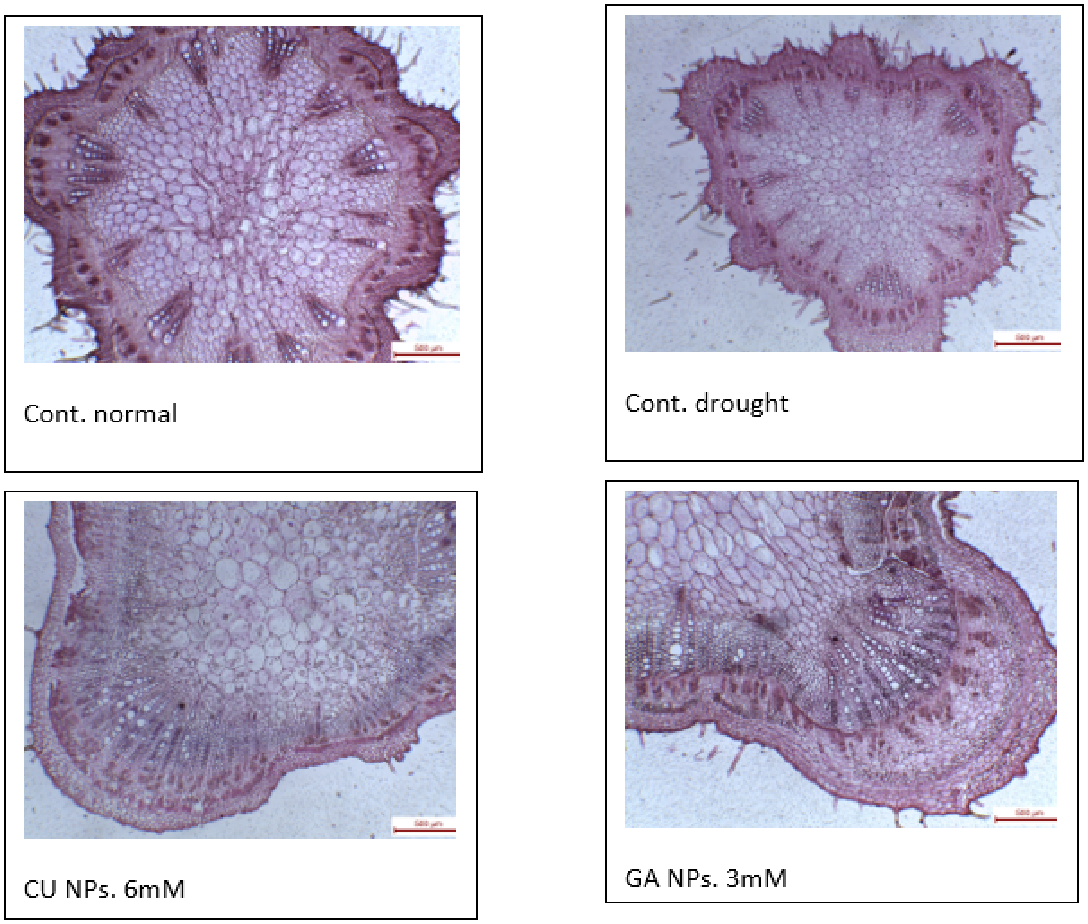



**Figure 7**

Transverse sections through the blade of soybean plant at the age of 90 days as sprayed with: 1- Control plant (100% FC). 2- Control plant (50% FC). 3- Plants sprayed with CU NPs at 3mM 4- Plant sprayed with GA NPs at 1mM (Details: Pal=palisade tissue, Spo= spongy tissue, Xy= xylem, Phl= phloem) 40X




### Figure 8

Transverse sections through the 4th internode of soybean stem at the age of 90 days as sprayed with: 1- Control plant (100% FC). 2- Control plant (50% FC). 3- Plants sprayed with curcumin at 6mM 4- Plant sprayed with glycyrrhizic acid at 3mM (Details: Epi=epidermis, Col= collenchymas tissue, Par=parenchyma tissue, Phl= phloem, Xy= xylem) 100X

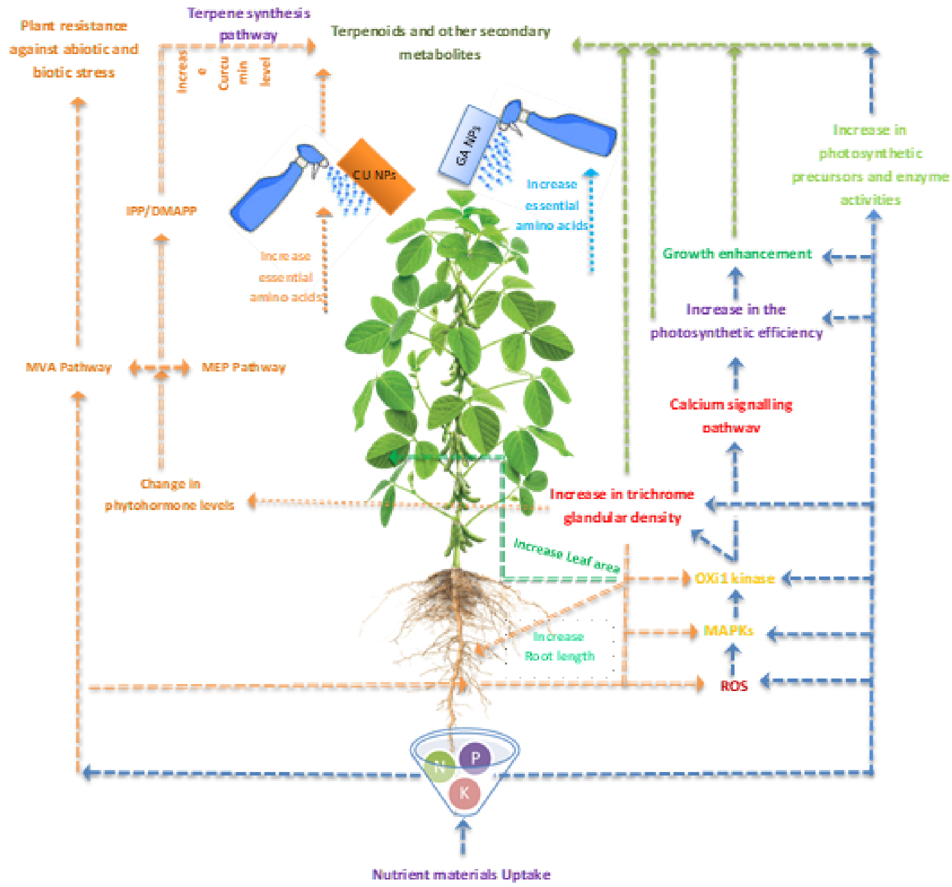



**Figure 9**

Schematic diagram for different physiological pathways in Soybean (Glycine max (L.) Merrill) plants under drought and normal conditions in response to exogenous applications of nanoglycyrrhizic acid and nanocurcumin.