PHYTOCHEMICAL STUDIES ON CELERY (APIUM GRAVEOLENS L.) PLANT UNDER USING CHEMICAL FERTILIZATION, BIOFERTILIZER AND THIDIAZURON TREATMENTS

M. K. Khalil 1, K. F. Taha 2, M.A.Nesem 1, S. S. Sallam* 2

1 Department of Agricultural Botany, Plant Physiology section, Faculty of Agriculture, Cairo University, Giza, Egypt.
2 Department of medicinal plant and natural products, National Organization for Drug Control and Research, Giza, Egypt.

*Corresponding author: saadshehab2011@yahoo.com

Abstract

A field experiment was conducted during two successive seasons of 2014/2015 and 2015/2016 at Phytochemistry Department and Farm of Applied Research Center of Medicinal Plants (ARCMP) affiliated to the National Organization for Drug Control And Research (NODCAR). Present work aimed to study the effect of inoculation Apium graveolens L. seeds with arbuscular mycorrhizal fungi (my) and/or microbein (mi) and/or foliar spray plants with thidiazuran (TDZ) combine with chemical fertilizer at half or full dose of NPK on the % of the volatile oil of celery seeds, physiochemical investigation (Specific gravity of each essential oil and Refractive index of each essential oil), Compositional analysis by GLC of essential oil content, total phenolic and flavonoid content of dry seeds. Were estimated the results showed that inoculation of celery (Apium graveolens L.) seeds with mixture of mycorrhizal and microbein at full dose of NPK gave the highest yield of the volatile oil and total phenolic and flavonoid content of dry seeds. While treated plants with biofertilizer (mycorrhizal and/or microbein) /or sprayed plants with (TDZ) combine biofertilizer at half or full dose of NPK didn’t have any significant effect on physiochemical investigation and compositional analysis of volatile oil by GLC as compared to the control.

Keywords: Apium graveolens, fertilization, biofertilizer, thidiazuron, fungi, microbein

Introduction

Celery (Apium graveolens L.) belongs to family apiaceae, and is one of the annual or perennial plants that grow throughout Europe and the tropical and subtropical regions of Africa and Asia (Gauri et al., 2015). Celery is an herbaceous annual or bioennial herb growing to a height of 60 to 90 cm. It has a shallow tape root system, the stem is branched succulent and ridged. The leaflets are ovate to sub orbicular three lobs 2-4.5cm. Long. The inflorescence is a compound umbel. The flowers are small and white and the calyx teeth are absolute. There are five petals ovate acute with in floured tips. The carpals are semi trade sub pentagonal the primary ridges are distinct and filiform. The fruit is a schizocarp, with two mericarps, sub-orbicular to ellipsoid; 1-2 mm in diameter, aromatic, and slightly bitter celery is naturally cross-pollinated but not self-incompatible (CDRI and Rastogi et al., 1994). Celery leaves consist of 88.0% moisture, 6.3% protein, 0.6% fat, 2.1% minerals, 1.4% fiber and 1.6% carbohydrates. Its mineral and vitamin contents are calcium, phosphorous, iron, carotene, riboflavin, niacin and vitamin C (Blish et al., 1972). The active constituents of celery plant were isoimperatorin, isoquercitrin, linoleic acid, magnesium, p-cymene, phosphorus, guaiacol, silicon. terpinene-4-ol, 3-N-butyl-phthalide, umbelliferone, vitamins A, C, B.
apiol, zinc. Volatile oil, containing d-limonene, with α-selinene, santalol, α and β eudesmol, dihydrocarvone. Phthalides, ligustilide, sedanolide, and sedanenolide. bergapten, isopimpinellin, apiumoside and celeroside (Garg et al., 1980). 3-buty1-4,5-dihydrophthalide, coumarins (seselin, ostenhol, apigravin, celerin), furanocoumarins (including bergapten), flavonoids (apigenin, apiin), phenolic compounds, choline and unidentified alkaloids. The essential oil contains delta limonene, various sesquiterpene. Celery is rich in betacarotene and folic acid.

A large number of beneficial microbes are present in tropical forest soils. The beneficial groups of microorganism commonly termed as biofertilizers, which include fungi, nitrogen fixing and phosphate solubilizing bacteria etc. mycorrhizal; very commonly arbuscular mycorrhizal (AM). AM fungi belongs to lower group of fungi and presently placed in the order Glomales (Moran and Benny, 1990).

Plant growth-promoting rhizobacteria (PGPR) that aggressively colonize plant roots and benefit plants by providing growth promoters. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on roots and shoots growth. Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seed germination, seedling health, plant vigor, plant height, shoot weight, nutrients content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes. PGPR are reported to influence the growth, yield, nutrients uptake by an array of mechanisms. They help in increasing nitrogen fixation in legumes, help in promoting free-living nitrogen-fixing bacteria, increase supply of other nutrients, such as phosphorus, sulphur, iron and copper, produce plant hormones, enhance other beneficial bacteria or fungi, control fungal and bacterial diseases and help in controlling insect pests (Saharan and Nehra, 2011).

Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea), is a synthetic diphenylurea (DPU) type cytokinin that is thought to encourage the synthesis and/or accumulation of purine type cytokinins (Thomas and Katterman, 1986). Khafaga and Abd-Elnaby (2007) on four wheat cultivars under different foliar application (0.1% ZnSO₄, 20 ppm paclobutrazol (pp 333), 2.0 ppb Thitiazuron (TDZ) and tab water) reported that, Sids1 cv. treated with TDZ produced the best development at tillering and harvesting stages. Concerning chemical composition, 2.0 ppb TDZ treatment enhanced proline content, photosynthetic pigments, total carbohydrates, protein, K⁺ and Ca²⁺ content in shoots of wheat plant as compared with the other treatments while the reverse was true for Na⁺ content.

The aim of the present work was to study the effect of mycorrhizal, microbein and TDZ combined with half or full dose of NPK on active constituents of celery plant.

MATERIALS AND METHODS

Plant material

Seeds of Apium graveolens L. family obtained from agriculture Research Center (EL gamma st., giza, Egypt).

MATERIALS

microorganisms material:-

1. mycorrhizal (contains Gloums spp., Gigaspora spp. and Acaulospora spp. V 1:1:1) obtained from soil, water and environment research institute.
2. microbein (biofertilizer containing N-fixing (such as Azotobacter and Azospirillum) and P-dissolving bacteria (Such as Pseudomonas and Bacillus megathéium) produced
and distributed commercially by the general organization for agriculture equalization fund. Ministry of Agriculture, Egypt.

**Mycorrhizal and microbein**

coated the seed of celery pre-planting by mixing with a fine mist of 10% sugar solution and mixing seed with the microbein and Mycorrhizal spores.

**Thiduazoran growth regulators**

Obtained from commercially compound named prop 50 WP (containing 50% TDZ).

Plants were sprayed with 10 ml of a solution containing (5 mg/l TDZ dissolving in water containing 0.01% tween 20%) using a hand atomizer. Weighing the plants before and after spraying showed that approximately 5 to 7 ml of the solution adhered to each plant. Control plants were sprayed with water containing 0.01% tween 20% but without TDZ.

**Soil used**

The soil used in the present work are collected from farm of Applied Research Center soil of Medicinal Plants (ARCMP) related to The National Orgnization for Drug Control And Research (NODCAR) and initially analyzed for chemical and physical characters according to Black et al. (1965). These characters are presented in Table (1).

**Table (1): Chemical and physical characteristics of experimental soil**

| EC mmohs/cm | SP | Ph | Ca++ | Mg++ | Na+ | K+ | CO3-- | HCO3-- | Cl- | SO4-- |
|------------|----|----|------|------|-----|----|-------|--------|-----|-------|
| 7.5        | 26 | 8.1| 8.10 | 9.32 | 2.57| .80| ----  | 2.6    | 4.24| 13.93 |

| Particle size distribution (%) | Texture class |
|------------------------------|---------------|
| Coarse sand | Fine sand | Silt | Clay | Sand clay |
| 47.15 | 23.17 | 19.91 | 9.77 | |

**Experimental design and layout**

The experiment was laid out in randomized block design (RBD) (6X7m) with 3 replications; each block was prepared to contain 10 rows. Randomization of the treatments was done with the help of random number table as advocated by Fisher, 1950. The treatments were:-

1) Control (untreated plants with chemical and bio-fertilizer)
2) full dose of chemical fertilizer.
3) full dose of chemical fertilizer + mycorrhizal
4) full dose of chemical fertilizer + microbein
5) full dose of chemical fertilizer + TDZ
6) full dose of chemical fertilizers + mycorrhizal + microbein
7) full dose of chemical fertilizer + mycorrhizal + TDZ
8) full doses of chemical fertilizers + microbein + TDZ
9) Half Recommended dose of chemical fertilizer.
10) Half recommended dose of chemical fertilizer + mycorrhizal
11) Half recommended dose of chemical fertilizer + microbein
12) Half recommended dose of chemical fertilizer + TDZ
13) Half recommended dose of chemical fertilizer + mycorrhizal + microbein
14) Half recommended dose of chemical fertilizer + mycorrhizal + TDZ
15) Half recommended doses of chemical fertilizer + microbein + TDZ

Recommended dose of chemical fertilizer were 200 Kg/Fadden superphosphate (12.5% P_{2}O_{5}) added before planting, while the plants were fertilizer with 200 Kg/Fadden ammonium sulphate (20.6 % N ) and 50 Kg/Fadden potassium sulphate (50% KO_{2} ) after 30 and 45 days from planting at two stages.

Extraction of essential oil

The essential oil from the seeds of *Apium graveolans* L. was extracted by steam distillation method as described previously by Mazari *et al.*, (2010). Steam containing the stored at a low temperature until further analysis.

1) Determination of the volatile oil %

volatile oil in extracted are determined by distilling the plant with water and glycerol, collected the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, measuring the volume of the oil in the distillate and calculating the percentage as volume to weight (v/w).

2) Physiochemical investigation

a) Specific gravity

Specific gravity of the essential oil was measured with a specific gravity bottle of 10 ml capacity. Following the acetone cleanse, the acetone fumes were removed by air blasts, and the specific gravity bottle was dried thoroughly. The specific gravity bottle was then filled with reference liquid, and its weight was measured on an analytical balance. Then, the specific gravity bottle was emptied, dried, filled with essential oil, and the weight was recorded accurately.

b) Refractive index

The refractive index of essential oil was determined with use of a Refractometer. The prism was opened washed with acetone and dried. A few drops of the essential oil were placed on the prism. The field of vision was divided into light and dark portions. The refractive index of essential oil was noted on the display.

3) Analysis of volatile oil by GLC

Gas chromatographic analysis of absolute oil of *Apium graveoleans* L. was carried out using HP Gas Chromatograph, Model G1530A. The analysis conditions were column Zebron (30mx0.25mmx.10 film thick ), initial oven temperature 60°C, final oven temperature 300°C. Both initial Injector and detector temperature were 275°C. Moisture free pure nitrogen at a flow rate of 10ml/minute was used as carrier gas. The constituents of essential oil of *Apium graveolans* L were identified by comparing their relative and absolute retention times with those of authentic standards. The Essential Oil composition was reported as a relative percentage of the total peak area.

2. Determination of phenolic and total flavonoid

Sample preparation

A ground dried sample of one gram was weighted phenolic and flavonoid contents were extracted with 50 mL 80% aqueous methanol on an ultrasonic bath for 20 minutes. An aliquot (2 mL) of the extracts was ultra centrifuged for 5 minutes at 14 000 rpm (Marinova, *et al.*, 2005).

Determination of total phenolic
The total phenolic content was determined by using the Folin-Ciocalteau assay. An aliquot (1 mL) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to 25 mL volumetric flask, containing 9 mL of distilled deionized water (add water). Reagent blank using dd water was prepared. One milliliter of Folin-Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 minutes, 10 mL of 7% Sodium carbonate solution was added to the mixture. The solution was diluted to volume (25 mL) with add water and mixed. After incubation for 90 minutes at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with Spectrophotometer. Data of total phenolic contents of celery are expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (mg GAE/g dw.). All samples were analyzed in triplicate (Marinova, et al., 2005).

**Determination of total flavonoid**

Total flavonoid content was measured by aluminum chloride colorimetric assay. An aliquot (1 ml) of extracts or standard solution of (+)-catechin (20, 40, 60, 80 and 100 mg/L) was added to 10 mL volumetric flask, containing 4 mL of water. To the flask was added 0.3 mL 5% sodium nitrite. After 5 minutes, 0.3 mL 10% aluminium chloride was added. At sixth minutes, 2 mL 1 M sodium hydroxide was added and the total volume was made up to 10 mL with add water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm with Spectrophotometer. Data of total flavonoid contents are expressed as milligrams of (+)-catechin equivalents (CE) per gram dry weight (mg CE/g dw). All samples were analyzed in triplicate (Marinova, et al., 2005).

**Statistical analysis:**

Data recorded on vegetative growth and chemical compositions were statistically analyzed, and separation of means was performed using the least significant difference (L.S.D.) test at the 5% level, as described by (Snedecor and Cochran, 1967).

**RESULTS AND DISCUSSIONS**

**a) Effect of chemical fertilization, bio-fertilizer and TDZ on the yield of the volatile oil of Apium graveolans L. plant**

Data concerning the effect of inoculation celery (Apium graveolans L.) seeds with biofertilizer (mycorrhizal and/or microbein) /or sprayed plants with TDZ combined with biofertilizer at half or full dose of NPK on the volume of the volatile oil (ml/kg dry seeds) are presented in Table (2).

Data showed that treated celery (Apium graveolans L.) with biofertilizer and /or TDZ at full recommended dose of NPK significantly increased yield of the volatile oil (ml/kg dry seeds) as compared to treated with biofertilizer and /or TDZ at half recommended dose of NPK.

Also the data showed that inoculation celery (Apium graveolans L.) seeds with mixture of mycorrhizal and microbein at full dose of NPK gave the highest yield of the volatile oil (ml/kg dry seeds) were (44 and 46) recorded in the first and second seasons respectively. On the other hand, the lowest yield of the volatile oil (ml/kg dry seeds) obtained with zero treatment in two seasons at those values were (27 and 29 ml/kg dry seeds) on celery (Apium graveolans L.) plant respectively.

These results agree with the finding of Hellal et al., (2011) showed that the highest values of (Anethum graveolens L.) oil yield content was recorded by the
treatment of bio-fertilizer plus 2/3 of recommended dose of nitrogen fertilizer. The highest essential oil yield of fennel (Foeniculum vulgare) plants was observed in combined bio-fertilizers + 50% NP (Dadkhah, 2012). In this connection, Singh et al. (2013) inoculated celery (Pimpinella anisum) plant with five levels of Bacillus circulans containing biofertilizer, i.e. 0, 10, 15, 20 and 25 kg/ha associated with 80 kg N +40 kg P₂O₅+ 40 kg K₂O/ha. The Bacillus circulans bio fertilizer containing 20 kg/ha registered higher seed yield of celery (15.10) kg/ha over control (11.40 kg/ha). The installment of Bacillus circulans containing bio fertilizer beyond 20 kg/ha confined to the further progress in celery production.

Similar results were recorded by El-Gendy et al. (2013) studied use of Biofertilizers (Nitrobin, Rhizobacteren and Microbein) with or without different doses of Urea in the cultivation of lemongrass (Cymbopogon citratus). Data clearly showed that the interaction treatments between of N and biofertilizers led to significant increase for yield of essential oil compared to control during both seasons. Applications of biofertilizers with nitrogen fertilizers resulted in a significant effect of Polyphenol and flavonoid content in both seasons. In this respect, Nejatzadeh-Barandozi (2014) they reported that the highest essential oil content of dill (Anethum graveolens L.) plant detected in biological fertilizer and chemical fertilizer. Identification of essential oil composition showed that content of carvone increased with application of Nitroxin biofertilizers. Overall, the beneficial bio-fertilizer combined with mineral fertilizer resulted in improving nutritional status and photosynthesis and thereby increase proportion of carbohydrates in the leaves which certainly reflected their effect on increase growth yield and physical or chemical fruit quality (Khalil, 2012).

b) Effect of chemical fertilization, bio-fertilizer and TDZ on Physiochemical investigation (Specific gravity and Refractive index) and Compositional analysis of volatile oil by GLC of celery (Apium graveolans L.) plant

The data in Tables (3-10) showed that treated biofertilizer (mycorrhizal and/or microbein) /or sprayed plants with TDZ combined with biofertilizer at half or full dose of NPK didn't have any significant effect on physiochemical investigation (Specific gravity and Refractive index) and compositional analysis of volatile oil percentage by GLC (limonene) as compared to the control.

c) Effect of chemical fertilization, bio-fertilizer and TDZ on total phenols of celery (Apium graveolans L.) seed

The effect of chemical, biofertilizer and TDZ on total phenolic content (mg/g seeds) in dry seeds of celery (Apium graveolans L.) plant, as in Table (11) indicated that inoculation celery (Apium graveolans L.) seeds with mixture of mycorrhizal and microbein at full recommended dose of NPK indicate significantly increased of total phenolic (mg/g seeds) content in dry seeds as compared to control.

The results at full dose of NPK in the first and second seasons respectively. On the other hand, the lower values were (2.95 and 5.59 mg/g) obtained by zero treatment in the first and second seasons, respectively.

Similar results were recorded by Aseri et al. (2008) they reported that, Inoculation Pomegranate (Punica granatum L.) with Azotobacter chroococcum , A. brasilense, Glomus mosseae and G. fasciculatum, had resulted in a significantly higher accumulation of total phenols in 4 months old inoculated plants.

El-Gendy et al. (2013) they reported that treated lemongrass (Cymbopogon citrates) with biofertilizers (nitrobein & rhizobacteria and microbein) with urea led to significant increment of polyphenol. In this connection, Seifi et al. (2014) they
mentioned that inoculation olive with using two arbuscular mycorrhizal fungi species including *Glomus mosseae* and *G. interaradices* led to significantly increased leaf total phenols. 

Salama *et al.* (2015) studied the effect of organic and bio-organic fertilizers on total phenolics (TPC), total flavonoids (TFC) and vitamin C as well as antioxidant activities of two sweet fennel cultivars Dolce and Zefa fino. The highest values of TPC, TFC and vit. C were recorded by Zefa fino cultivar when received 50% NPK +50% organic treatment. On the other hand, the positive benefit that derived through using Azotobacter or AM singly and in dual inoculation integrated with mineral N or P fertilizer on chemical fruit quality criteria was discussed by (El-Khawaga and Maklad, 2013) and (Khehra, 2014) on citrus, (Sharma *et al.*, 2013) on guava, (Kundu *et al*. 2011) on mango, (Singh *et al.*, 2011) on apple and (Rueda *et al.*, 2016) on strawberry.

**Effect of chemical fertilization, bio-fertilizer and TDZ on total flavonoid of celery (*Apium graveolans* L.) seed**

Data listed in Table (12) showed that the total flavonoid content (mg/g) was increased in seeds resulting from inoculation celery (*Apium graveolans* L.) seeds with biofertilizer and/or foliar plants with TDZ at full recommended dose of NPK as compared to control in all treatments.

Also the data showed that the highest values of total flavonoid content (mg/g seeds) in dry seeds of celery (*Apium graveolans* L.) were (2.21 and 2.45 mg/g) obtained by inoculation celery (*Apium graveolans* L.) seeds with mixture of mycorrhizal and microbein at full dose of NPK in the first and second seasons respectively. On the other hand, the lower values were (1.0 and 1.03 mg/g) obtained by zero treatment in the first and second seasons, respectively.

These results were in harmony with the finding by El-Gendy *et al.* (2013) they showed that treated lemongrass (*Cymbopogon citrates*) with biofertilizer (nitrobein & rhizobacteria and microbein) with urea led to significant effect of flavonoid content in both seasons. This agreed with the result obtained by (Faramawy., 2014) reported that inoculation *Prosopis chilensis* with *Bradyhizobium japonicum*, *Azotobacter chroococcum*, *Bacillus megatherium* and VA mycorrhizal led to significantly increased total flavonoids.

| Treatment          | Growing season | 2014-2015 | 2015-2016 | Mean |
|--------------------|----------------|-----------|-----------|------|
| Zero               |                | 34        | 35        | 34.5 |
| full dose          |                | 39        | 39        | 39   |
| full + my          |                | 35        | 39        | 37   |
| full + TDZ         |                | 42        | 44        | 43   |
| full + my + mi     |                | 42        | 42        | 42   |
| full + my + TDZ    |                | 40        | 40        | 40   |
| full + mi + TDZ    |                | 44        | 46        | 45   |
| half dose          |                | 21        | 21        | 21   |
| half + my          |                | 24        | 28        | 26   |
| half + mi          |                | 24        | 24        | 24   |
| half + TDZ         |                | 22        | 26        | 24   |
| half + my + mi     |                | 30        | 34        | 32   |
| half + my + TDZ    |                | 30        | 30        | 30   |
| half + mi + TDZ    |                | 27        | 29        | 28   |
| Treatment          | Growing season | 2014-2015 | 2015-2016 | Mean |
|--------------------|----------------|-----------|-----------|------|
| Zero               |                | 0.975     | 0.981     | 0.978|
| full dose          |                | 0.995     | 0.991     | 0.993|
| full + my          |                | 0.975     | 0.995     | 0.985|
| full + mi          |                | 0.984     | 1         | 0.992|
| full + TDZ         |                | 0.978     | 0.987     | 0.983|
| full + my + mi     |                | 0.994     | 0.999     | 0.997|
| full + my + TDZ    |                | 0.981     | 0.983     | 0.982|
| half dose          |                | 0.99     | 0.992     | 0.991|
| half + my          |                | 0.997     | 0.98      | 0.989|
| half + mi          |                | 0.982     | 0.982     | 0.982|
| half + TDZ         |                | 0.994     | 0.984     | 0.989|
| half + my + mi     |                | 0.993     | 0.995     | 0.994|
| half + my + TDZ    |                | 0.981     | 0.988     | 0.985|
| half + mi + TDZ    |                | 0.985     | 0.985     | 0.985|
| Mean               |                | 0.987     | 0.989     |      |
| L.S.D.0.05         |                | NS        | NS        |      |

**Table 3.** Effect of chemical fertilization, biofertilizer and TDZ on mean specific gravity of volatile oil in dry seeds of celery plant during 2014/2015 and 2015/2016 seasons.

| Treatment          | Growing season | 2014-2015 | 2015-2016 | Mean |
|--------------------|----------------|-----------|-----------|------|
| Zero               |                | 1.555     | 1.56      | 1.558|
| full dose          |                | 1.561     | 1.56      | 1.561|
| full + my          |                | 1.556     | 1.555     | 1.556|
| full + mi          |                | 1.555     | 1.557     | 1.556|
| full + TDZ         |                | 1.56      | 1.448     | 1.504|
| full + my + mi     |                | 1.559     | 1.560     | 1.560|
| full + my + TDZ    |                | 1.557     | 1.552     | 1.555|
| full + mi + TDZ    |                | 1.557     | 1.558     | 1.558|
| half dose          |                | 1.558     | 1.554     | 1.556|
| half + my          |                | 1.562     | 1.561     | 1.562|
| half + mi          |                | 1.554     | 1.556     | 1.556|
| half + TDZ         |                | 1.562     | 1.555     | 1.559|
| half + my + mi     |                | 1.558     | 1.556     | 1.557|
| half + my + TDZ    |                | 1.557     | 1.57      | 1.564|
| half + mi + TDZ    |                | 1.561     | 1.56      | 1.561|

**Table 4.** Effect of chemical fertilization, biofertilizer and TDZ on mean refractive index of volatile oil in dry seeds of celery plant during 2014/2015 and 2015/2016 seasons.
### Table 5. Effect of chemical fertilization, biofertilizer and TDZ on mean limonene % of volatile oil in dry seeds of celery plant during 2014/2015 and 2015/2016 seasons.

| Treatment          | Growing season |          |          |          |
|--------------------|----------------|----------|----------|----------|
|                    | 2014-2015      | 2015-2016| Mean     |          |
| Zero               | 87.64          | 87.1     | 87.37    |          |
| full dose          | 88.17          | 88.64    | 88.41    |          |
| full + my          | 86.42          | 87.25    | 86.84    |          |
| full + mi          | 87.94          | 87.5     | 87.72    |          |
| full + TDZ         | 87.98          | 87       | 87.49    |          |
| full + my + mi     | 86.79          | 86.93    | 86.86    |          |
| full + my + TDZ    | 86.18          | 87.37    | 86.78    |          |
| full + mi + TDZ    | 87.28          | 88.4     | 87.84    |          |
| half dose          | 88.9           | 88       | 88.45    |          |
| half + my          | 88.76          | 88.97    | 88.87    |          |
| half + mi          | 88.95          | 88.45    | 88.70    |          |
| half + TDZ         | 88.12          | 87.9     | 88.01    |          |
| half + my + mi     | 88             | 88.41    | 88.21    |          |
| half + my + TDZ    | 87.35          | 88       | 87.68    |          |
| half + mi + TDZ    | 88.9           | 88       | 88.45    |          |
| Mean               | 87.83          | 87.86    |          |          |

L.S.D.0.05           | NS             | NS       |          |          |

### Table 6. Effect of chemical fertilization, biofertilizer and TDZ on mean total phenolic content (mg/g) in dry seeds of celery plant during 2014/2015 and 2015/2016 seasons.

| Treatment          | Growing season |          |          |          |
|--------------------|----------------|----------|----------|----------|
|                    | 2014-2015      | 2015-2016| Mean     |          |
| Zero               | 3.65           | 4.34     | 4.00     |          |
| full dose          | 4.3            | 5.17     | 4.74     |          |
| full + my          | 4.27           | 5.60     | 4.94     |          |
| full + mi          | 4.2            | 5.37     | 4.79     |          |
| full + TDZ         | 5.4            | 6.07     | 5.74     |          |
| full + my + mi     | 4.95           | 5.59     | 4.27     |          |
| full + my + TDZ    | 3.2            | 5.65     | 4.43     |          |
| full + mi + TDZ    | 3.16           | 4.80     | 3.98     |          |
| half dose          | 2.9            | 5.12     | 4.01     |          |
| half + my          | 3.6            | 5.00     | 4.30     |          |
| half + mi          | 3.45           | 4.77     | 4.11     |          |
| half + TDZ         | 3.8            | 5.35     | 4.58     |          |
| half + my + mi     | 4              | 4.45     | 4.23     |          |
| half + my + TDZ    | 4              | 5.18     | 4.59     |          |
| half + mi + TDZ    | 3.95           | 4.99     | 4.47     |          |
| Treatment          | Growing season | 2014-2015 | 2015-2016 | Mean | L.S.D.0.05 |
|--------------------|----------------|-----------|-----------|------|------------|
| Zero               |                | 0.95      | 1.02      | 0.99 |            |
| full dose          |                | 1.77      | 2.7       | 2.23 |            |
| full + my          |                | 1.3       | 2.11      | 1.71 |            |
| full + mi          |                | 0.93      | 2.7       | 1.82 |            |
| full + TDZ         |                | 3.2       | 1.9       | 2.55 |            |
| full + my + mi     |                | 2.8       | 2.33      | 2.57 |            |
| full + my + TDZ    |                | 1.2       | 2.24      | 1.72 |            |
| full + mi + TDZ    |                | 3.2       | 2.16      | 2.68 |            |
| half dose          |                | 0.87      | 1.1       | 0.99 |            |
| half + my          |                | 2.27      | 1.3       | 1.79 |            |
| half + mi          |                | 1.78      | 1.34      | 1.56 |            |
| half + TDZ         |                | 0.9       | 1.15      | 1.03 |            |
| half + my + mi     |                | 2.1       | 1.57      | 1.84 |            |
| half + my + TDZ    |                | 1.9       | 1.94      | 1.92 |            |
| half + mi + TDZ    |                | 2.1       | 1.38      | 1.74 |            |
| Mean               |                | 1.89      | 1.56      | 1.82 | 0.16       |
| L.S.D.0.05         |                | 0.16      | 0.18      |      |            |

**Table 7. Effect of chemical fertilization, biofertilizer and TDZ on mean total flavonoides content (mg/g) in dry seeds of celery plant during 2014/2015 and 2015/2016 seasons.**

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أجريت تجربتان حفظيان بمزرعة مركز الدراسات التطبيقية لبحوث النباتات الطبية التابع للهيئة القومية للرقابة والباحث الدوائي—الهيئة القومية للرقابة والبحث الدوائي—الجيزة، جمهورية مصر العربية

أثناء تجربتي، تم تدوير النباتات بالميكروبيهما والميكروبيهما بالتمثيل الحيوي (النيتروجين والفسفور والنيتروجين والميكروبيهما) واستخدام نصف الرمية المصممة بها TDZ النباتات الفعاله في نبات الكرفس الافرنجي TDZ النباتات الفعاله في نبات الكرفس الافرنجي

أظهرت تجربتي، أن تأثير الميكروبيهما والميكروبيهما بالتمثيل الحيوي (الميكروبيهما والميكروبيهما) TDZ النباتات الفعاله في نبات الكرفس الافرنجي TDZ النباتات الفعاله في نبات الكرفس الافرنجي