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# Tolerance of bio-fertilized *Delonix regia* seedlings to irrigation intervals

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**This work aims to investigate the effect of different bio-fertilizers (*Arbascular mycorrhizae* fungi, *Azotobacter chroorcoccum*, yeast strains and mixture of all inoculum) and irrigation intervals (3, 6 or 12 days) on the growth and chemical composition of *Delonix regia* seedlings grown in sandy soil. Pot experiments were conducted using a randomized complete blocks design with three replicates during two successive seasons of 2013 and 2014. The results indicated that dual bio-fertilizers led to significant increase in growth characters (plant height, root length, number of branches/plant, total fresh and dry weights/plant), microbial populations and AM fungi colonization (%), enzymatic activities, chemical composition (plant pigments, total carbohydrates, proline content, N, P, K) besides antioxidant enzymes such as catalase (CAT),and peroxidase (POD) compared to the un-inoculated seedlings (as control) at the recommended dose of NPK chemical fertilizers under the same conditions. Generally, these results undoubtedly confirm that dual bio-fertilizers could replace the use of chemical fertilizers and consequently improve the quality and quantity of *D. regia.***

**Key words:** *Delonix regia*, *Arbascular mycorrhizae* fungi, *Azotobacter chroorcoccum*, yeast strains - growth characters, chemical composition.

## INTRODUCTION

Lack of fresh water resources for agriculture is the most important problem facing many countries in arid and semi-arid regions of Africa, such as Egypt. Thus there is a need to look for alternative methods to balance sustenance with demand (Wolters et al., 2013).

Using drought-tolerant trees in dry regions is one of many ways used to solve this problem. *Delonix regia* is one of the most important and common drought tree species in Egypt which also tolerates a wide variety of soils and conditions but needs to be well-watered until it gets established. The genus name is derived from the Greek words delos (meaning conspicuous) and onyx (meaning claw) referring to the appearance of the spectacular flowers. The tree is commonly cultivated in the tropics and subtropics, including Madagascar (Menninger, 1962). It plays a key role in regulating

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climate, resisting wind, sand, conserving water and soil (Du Puy et al., 2002). It is an ornamental tree found in streets and parks. It is fast-growing and develops an umbrella-shaped crown, making it a valuable shade tree. The wood is widely used as firewood and for making fence posts. It has an antioxidant potential (Auudy et al., 2003) and its seeds contain gum that may be used in food and textile industries. Its dried seeds can also be used as a binder in the manufacturing of tablets (e.g. Paracetamol). Its bark has medicinal properties (Little and Wadsworth, 1964; Webb et al., 1984).

Numerous studies have found that plants have mechanisms to cope with drought stress; they will become more tolerant to drought when associated with different soil microorganisms (Soliman, 2008; Aroca and Ruiz-Lozano, 2009). Beneficial microorganisms are a tool that enhances yield, plant growth and nutrient uptake under various environmental conditions such as salinity, drought and low fertility supply. Some endomycorrhizal fungi (*Arbuscular mycorrhizal* fungi) have been proven to improve drought stress; they colonize bio-trophically the root cortex and develop an extra-metrical mycelium that helps the plants to acquire mineral nutrients from the soil particularly those, which are immobile. They can under drought conditions stimulate growth-regulating substances, increase photosynthesis, improve osmotic adjustment, optimize hormonal balance and enhance water uptake (Colla et al., 2007).

Some yeast species (*Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa*) and *Azotobacter* spp. have evolved different strategies to adapt with the changes in their environment. They can combat high osmolarity by enhancing transcription (Treuner-lange et al., 1997) or by the presence of some stress enzymes like catalases and peroxidases or organic and inorganic compounds.

Therefore, this study aims to investigate the effect of specific bio-fertilizers on increasing the drought resistance of *D. regia* seedlings under irrigation intervals.

## MATERIALS AND METHODS

The present study was carried out at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and Studies, Cairo University, and at the Microbiology Department, Soils, Water and Environment Institute (SWERI), Agricultural Research Center, Giza, Egypt, during the two successive seasons of 2013 and 2014.

### Plant material

Seeds of *D. regia* (Bojer ex Hook.) Raf. were obtained from the Faculty of Agriculture, Giza, Egypt. They were soaked in hot water (90°C) for 10 s followed by 24-h imbibitions, to accelerate germination (Millat and Mustafa, 1989). On the first of June, the seeds were then sown in 8-cm plastic pots filled with sandy soil. After two weeks, in both seasons, the seedlings (15 cm tall) were transplanted into plastic pots (30 cm diameter) filled with 6 kg of the same sandy soil. The physical and chemical characteristics of the soil are shown.

### Soil analysis

The soil texture was sandy having the following characteristics: Coarse sand, 30.82%; fine sand, 62.61; silt, 1.22%; clay, 5.35%; pH, 7.75; EC, 1.15 ds/m; organic matter, 0.08%; available N, 6.9 (ppm); available P, 6.2 (ppm); available K, 64 (ppm); $CaCO_3$, 0.26%; and water holding capacity, 14.5%.

### Treatments

At the beginning of the experimental, pots were supplied with recommended dose of NPK at a rate of 2.1 g/pot ammonium sulphate (20.5%) as nitrogen. Phosphorus was added as superphosphate (15.5%) at a rate of 1.2 g/pot and potassium was added as potassium sulphate at a rate of 0.3 g/pot. The seedlings were divided into seven treatments. The first one was un-inoculated control plants whereas the second treatment received only full dose of NPK after one month of each season. In the third treatment, the plants inoculated with mixed spores of AMF from genera (*Glomus*, *Gigaspora*and *Acaulospora*) (500 spores/g) at a rate of 3 g/hole, where spores dressed in a hole around the rhizosphere were attached to secondary roots (Massoud et al., 2009). Once the mycorrhizal symbiosis was established, *A. chroococcum*as a fourth treatment was prepared by growing on modified Ashby's medium $10^8$ CFU/ml for 5 days (Abdel Malak and Ishac, 1968); whereas yeast strains as fifth and sixth treatments (*S. cerevisiae* and *R. mucilaginosa*) were also incubated at 28°C on rotary shaker at 150 rpm for 48 h in conical flasks (250 ml) containing 100 ml of glucose peptone yeast (GPY) medium (Difco, 1985). Then both were individually applied monthly at a rate of 5 ml/ pot. In addition, the mixture of previous inoculums (AMF, *A. chroococcum*, yeast strains) as seventh treatment was inoculated; it was isolated from very dry soil located at Tushka Valley Region, where there are great variations between the temperatures at day and night in winter and summer.

### Irrigation intervals

The plants were irrigated every 3, 6, or 12 days. At each irrigation, the plants were watered till 100% of field capacity (F.C.). The soil moisture tension was measured before each irrigation using micro tension meters, and the quantity of water needed to reach 100% F.C. was calculated, as described by Richards (1949).

### Experimental design

This experiment was factorial, conducted using a randomized complete blocks design with three replicates. The study included 21 treatments [7 treatments×3irrigation intervals], with each block consisting of 105 plants (5 plants/treatment).The treatments were applied regularly until the termination of each season (1$^{st}$ September in both seasons).

### Growth parameters

Plant height, root length, number of branches/plant, as well as total fresh and dry weights /plant were recorded.

### Microbial populations and AM fungi colonization

The population dynamics of total bacterial and yeast count in the rhizospheric zone of *D. regia* roots was determined by the plate count technique according to Reinhold et al. (1985). While

*Azotobacter* spp. population counts in the rhizospheric zone of *D. regia* roots were determined using the most probable number (CFU/g rhizosphere) method described by Cochran (1950).

The percentage of AM fungi colonization in plant root tissues was also determined as described by Phillips and Hayman (1970).

**Enzymatic activities determinations**

Nitrogenase activity ($N_2$-ase) in rhizosphere (roots) was measured as described by Somasegaran and Hoben (1994). The dehydrogenase activity was also estimated according to Skujins and Burns (1976).

**Photosynthetic pigments, total carbohydrates and proline determinations**

In addition, chemical analysis of fresh leaves samples was conducted to determine total chlorophyll (a+b) and carotenoids contents, using the method described by Nornai (1982). The content of total carbohydrates in dried leaves samples was determined using the method described by Dubois et al. (1956). The proline content in fresh leaves was also determined according to Bates et.al. (1973).

**Determination of elements**

Dried leaves samples were digested to extract nutrients as described by Piper (1950), and the extract was analyzed to determine its contents of nitrogen (using the modified micro-Kjeldahl method as described by Pregl (1945), phosphorus according to Jackson (1967) and potassium estimated photometrically using a Jenway flame photometer, according to Chapman and Pratt (1961).

**Activities of antioxidant enzymes**

Preparation of the enzymes extraction of leaves tissues was carried out at 40°C at 3:1 buffer: fresh weight (v/v) in a pastel and mortar with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 rpm for 30 min and then the supernatants were stored in separate aliquots at 8°C (Vitoria et al., 2001). Antioxidant enzymes were assayed as follows; Catalase (CAT) by measuring the decrease in absorbance due to disappearance of $H_2O_2$at 240 nm according to Chance and Maely (1955), Peroxidase (POD) by spectrophotometry according to Amako et al. (1994). Enzymes activities were expressed as units/min/mg protein.

All the obtained data were subjected to statistical analysis of variance, and the means were compared using the "Least Significant Difference (L.S.D.)" test at the 5% level, as described by Little and Hills (1978).

## RESULTS AND DISCUSSION

### Growth parameters

The obtained results revealed that, the prolonged irrigation intervals had an adverse effect on the growth of *D. regia* plants, regardless of the effect of inoculation treatments (Table 1). In both seasons, prolonging irrigation intervals (12 days) steadily resulted in significant reduction in the values recorded for all of the growth parameters (plant height, root length, number of branches/plant, as well as total fresh and dry weights /plant). However, prolonged irrigation intervals from 3 to 6 days caused only a slight (insignificant) reduction in the mean values recorded for some studied growth parameters, whereas longer irrigation intervals (12 days) resulted in significant reduction in the values recorded for all of the vegetative characteristics.

It can be concluded that the reduction in the growth may be attributed to the participation of water in the cell division, cell expansion and cell enlargement. In addition, water stress reduction causes a decrease in transport of cytokinin from root to shoot and/or increase in Abscisic acid in leaf; these changes in the balance of hormones cause change in the extensibility in cell wall and these affect generally growth enlargement (Siddique et al., 2000; Ouma, 2005; Luvaha, 2005). This result has also been confirmed by Oyun et al. (2010) on *Acacia senegal*, and Liu et al. (2011) on apple.

The growth parameters of *D. regia* under different treatments as shown in Table 1 undoubtedly revealed that seedlings inoculated with dual bio-fertilizers significantly showed higher values in growth parameters compared to un-inoculated (control) plants at the recommended dose of NPK under the same conditions, in both seasons. Total fresh weight/plant was increased by 60.01 and 59.24% in the first and second seasons, respectively, over control plants.

These results provide a plausible mechanism of how the dual bio-fertilizers led to increase in growth parameters in the control. This rise of their beneficial effects on seedlings represented in tolerance to drought produces some growth promoting substances (Gibberellins, IAA and Abscisic acid, etc.), and vitamins which have favorable effects on root development (Alexander, 1977; Dobbelaere et al., 2003). Hyphae produced by AM fungi, which are microscopic tubes, colonize plant roots and grow out into the soil further than root hairs. The hyphae help in retaining moisture around the root zone of plants, and also increase nutrients uptake to the plant (especially diffusion of limited nutrient like P). Moreover, yeast contents of micro and macronutrients stimulate the plant to build up dry. In addition, they reduce diseases caused by root pathogens matter (Morte et al., 2001; Ortas et al., 2002; Hesham and Mohamed, 2011). Supportive evidence for this view was reported by Ibrahim (2009) on Flame seedless and Superior grapevines and Ibrahim et al. (2010) on Balady guava trees.

### Microbial populations, mycorrhizal colonization (%), and enzyme activities

Data presented in Figures 1 and 2 also revealed that prolonged irrigation intervals had an adverse effect on the mean number of microbial populations (total microbial,

**Table 1.** Influence of bio-inoculants and irrigation intervals on the growth parameters of *D. regia* during 2013 and 2014.

| Treatment (T) | Irrigation intervals (I) | Growth parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | | Root length (cm) | | Stem diameters (cm) | | Number of branches/plant | | Total fresh weight (g/ plant) | | Total dry weight (g/plant) | |
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| **Control** | | 46.20 | 42.00 | 54.00 | 50.67 | 1.00 | 0.70 | 8.00 | 5.00 | 63.33 | 58.83 | 29.67 | 26.42 |
| Full NPK | | 86.33 | 84.23 | 88.00 | 83.00 | 1.40 | 1.10 | 9.33 | 7.00 | 95.26 | 89.98 | 45.30 | 43.99 |
| AMF | | 100.27 | 99.00 | 106.67 | 101.33 | 2.10 | 1.70 | 14.33 | 13.67 | 103.89 | 97.83 | 50.28 | 47.92 |
| *Azotobacter chroococcum* | 3 Days | 91.17 | 88.00 | 95.00 | 92.33 | 1.70 | 1.30 | 11.33 | 9.00 | 97.40 | 91.39 | 47.04 | 44.03 |
| *Rhodotorula mucilaginosa* | | 83.10 | 79.00 | 83.00 | 80.00 | 1.30 | 1.05 | 8.33 | 7.00 | 91.19 | 86.72 | 44.60 | 41.03 |
| *Saccharomyces cerevisiae* | | 95.00 | 91.73 | 102.33 | 100.00 | 1.90 | 1.40 | 12.00 | 10.67 | 99.61 | 94.35 | 48.81 | 45.84 |
| Mixture of all | | 108.50 | 42.00 | 109.33 | 105.67 | 2.40 | 2.00 | 16.33 | 14.33 | 105.53 | 99.30 | 51.43 | 49.65 |
| **Control** | | 87.22 | 84.15 | 48.33 | 42.33 | 0.80 | 0.60 | 7.00 | 3.67 | 59.04 | 51.63 | 26.19 | 23.48 |
| Full NPK | | 40.33 | 38.00 | 70.67 | 66.33 | 1.23 | 0.80 | 8.00 | 5.00 | 82.07 | 72.65 | 39.04 | 34.33 |
| AMF | | 78.00 | 73.33 | 91.33 | 90.00 | 1.90 | 1.40 | 12.67 | 10.33 | 97.65 | 88.02 | 46.16 | 41.36 |
| *A. chroococcum* | 6 Days | 97.80 | 95.00 | 83.67 | 81.67 | 1.60 | 1.10 | 9.67 | 6.67 | 90.55 | 80.59 | 43.61 | 37.63 |
| *R. mucilaginosa* | | 85.00 | 82.13 | 74.33 | 70.00 | 1.40 | 1.00 | 8.33 | 5.33 | 87.26 | 75.80 | 41.97 | 35.90 |
| *S. cerevisiae* | | 80.67 | 77.00 | 88.00 | 85.00 | 1.70 | 1.30 | 11.33 | 8.00 | 94.75 | 83.85 | 45.71 | 40.89 |
| Mixture of all | | 89.00 | 95.00 | 95.00 | 92.67 | 2.00 | 1.60 | 15.33 | 12.00 | 98.35 | 90.55 | 47.51 | 43.28 |
| **Control** | | 29.40 | 26.8 | 33.00 | 29.33 | 0.60 | 0.30 | 4.67 | 2.33 | 39.76 | 37.46 | 16.88 | 14.73 |
| Full NPK | | 59.67 | 54.13 | 67.33 | 64.00 | 0.80 | 0.60 | 5.33 | 3.67 | 74.35 | 67.64 | 35.18 | 29.82 |
| AMF | | 82.00 | 80.67 | 86.33 | 80.00 | 1.25 | 1.14 | 10.67 | 8.00 | 91.56 | 85.80 | 45.11 | 40.57 |
| *A. chroococcum* | 12 Days | 71.00 | 66.50 | 75.00 | 73.67 | 1.09 | 1.03 | 7.33 | 5.33 | 85.80 | 77.63 | 40.23 | 35.82 |
| *R. mucilaginosa* | | 66.17 | 61.80 | 70.00 | 68.33 | 1.00 | 0.90 | 6.00 | 4.00 | 80.25 | 72.12 | 36.79 | 34.06 |
| *S. cerevisiae* | | 74.90 | 68.73 | 82.33 | 76.67 | 1.19 | 1.10 | 9.00 | 6.67 | 88.90 | 81.47 | 42.12 | 39.74 |
| Mixture of all | | 89.10 | 86.17 | 89.33 | 84.33 | 1.50 | 1.20 | 11.67 | 9.33 | 93.92 | 86.87 | 45.63 | 42.10 |
| **LSD (0.05)** | | | | | | | | | | | | | |
| I | | 2.30 | 2.00 | 5.82 | 3.77 | 0.32 | 0.18 | 2.40 | 0.94 | 5.05 | 5.53 | 2.91 | 4.24 |
| T | | 2.49 | 2.60 | 6.63 | 7.48 | 0.24 | 0.24 | 0.87 | 0.74 | 5.67 | 9.36 | 2.99 | 4.80 |
| I X T | | 4.32 | 4.50 | 11.48 | 12.96 | 0.42 | 0.41 | 1.51 | 1.28 | 9.82 | 16.22 | 5.19 | 8.32 |

*Azotobacter* spp. and yeasts count), mycorrhizal colonization (%), as well as enzyme activities (nitogenase and dehydrogenase activities), regardless of the effect of inoculation treatments. In both seasons, prolonged irrigation intervals steadily reduced the values recorded for these parameters. This reduction can be attributed to the role of water in enhancing the microbial activities (Ouma, 2007). Preceding results are in harmony with those obtained by Soliman (2008) on *Acacia nilotica*, who reported that the nitrogenase and dehydrogenase activities were decreased with

prolonged irrigation intervals.

In general, microbial population, mycorrhizal colonization (%) and enzyme activities in the rhizosphere of *D. regia* were significantly affected by dual bio-fertilizers, as compared to the control which had the highest microbial populations, mycorrhizal colonization (%), as well as enzyme activities (Figures 1 and 2). Many studies have shown that the power of dual bio-fertilizers is due to their production of secondary metabolites that are essential for the growth of almost all the microorganisms, Nitrogenase and other proteins involved in nitrogen fixation (Brill, 1980; Muthuselvan and Balagurunathan, 2013). In addition, AMF development could be enhanced by supplying yeast vitamin B12, as it stimulates it (Boby et al., 2008). Also, AM fungi stimulate the activity of beneficial soil microorganisms (Boby and Bagyaraj, 2003) and root exudation is modified both qualitatively and quantitatively by *A. mycorrhizal* symbiosis. This leads to increase in mycorrhizal infection (Garg and Manchanda, 2009). Supportive evidence for this view was reported by Harisudan et al. (2010).

## Chemical composition

### *Total chlorophyll and carotenoids content*

Data recorded in the two seasons (Table 2) revealed that prolonged irrigation intervals had an adverse effect on the total chlorophyll and carotenoids contents in the leaves of *D. regia* plants, regardless of the effect of inoculation treatments. In both seasons, the contents of total chlorophyll and carotenoids were reduced steadily as the irrigation intervals were prolonged daily to 6 or 12 days. Drought stress causes reduction of the $CO_2$ concentration in leaf internally. This is a result of stomata closure, changes in chlorophyll content, chlorophyll components and damage of the photosynthetic apparatus. All these led to the reduction rates of leaf photosynthetically. Also, producing reactive oxygen species (ROS) such as $O_2$- and $H_2O_2$ can lead to lipid peroxidation and consequently, chlorophyll destruction (Foyer et al., 1994; Iturbe Ormaetxe et al., 1998; Lawlor and Cornic 2002; Flexas et al., 2004). Similar reductions in the chlorophylls content were reported by Mafakheri et al (2010), on *Cicer arietinum* and Arjenaki et al (2012) on *Triticum aestivum*.

Data presented in Table 2 also revealed that in both seasons, the total chlorophyll and carotenoids contents were affected by dual bio-fertilizers, as compared to the control plants. Plants inoculated with the dual bio-fertilizers had the highest total chlorophyll and carotenoids contents, followed by plants inoculated with AMF, then un-inoculated plants, in both seasons. These augmentations in the total chlorophyll and carotenoids content were in the favor of control plants which recorded 69.78 and 66.88%, respectively.

Similar results have been reported by El-Khateeb et al. (2011) who stated that chlorophyll and carotenoids content in *Acacia saligna* were improved by inoculation with A. mycorrhizal fungi under water stress; also, Mazhar et al. (2010) on *Jatropha curcas*. The promotion of the synthesis and accumulation of chlorophyll may be attributed to the dual inoculation of AM fungi with other beneficial microorganisms that enhance mineral nutrition such as N, which is an essential component in the structure of porphyrines, which are found in many metabolic active compounds, including chlorophylls. And also, the role of cytokine yeast that delays the aging of leaves. It does this by reducing the degradation of chlorophyll, leading to increase in chlorophyll content. Thus, it helps in higher photosynthetic rate (Castelfranco and Beale, 1983; Feng et al., 2002; Boby et al., 2008).

### *Total carbohydrates percentage and proline content*

The data in Table 2 also showed that, in both seasons, prolonged irrigation intervals steadily increased the total carbohydrates percentage and proline content. These increments were 38.10 and 41.18%, respectively over control plants.

This behavior may be attributed to a reduction of carbohydrates translocation from leaves to other plant parts under drought conditions and/or the lesser consumption of carbohydrates in the leaves (El-Khateeb et al., 1991). Also, Hoekstra et al. (2001) mentioned that a high carbohydrate concentration decreases water potential, contributes in preventing oxidative damage, and maintains the structure of proteins and membranes under moderate dehydration during drought period.

The increase in the proline content of plants irrigated at long intervals is in agreement with the findings of El-Quesni et al. (2012) who reported that the proline content in leaves, stems and roots of *Matthiola incana* significantly increased as a result of decreased soil moisture level. This confirms that proline can biochemically adapt to stress condition.

The mean total carbohydrates percentage and proline content in leaves of *D. regia* were affected by dual bio-fertilizers (Table 2). In both seasons, plants inoculated with dual bio-fertilizers had the highest total carbohydrates percentage and proline content, followedby plants inoculated with AMF, *S. cerevisiae*, *A. chroococcum* and *R. mucilaginosa*, full NPK, and then un-inoculated plants. The favorable effect of biofertilization on the synthesis and accumulation of carbohydrates and proline may be attributed to the increase in the chlorophylls content of inoculated plants, and to the role played by nitrogen in the structure of porphyrine molecules (as previously mentioned), which are found in the cytochrome enzymes essential in photo-synthesis. This increase in the contents of chlorophylls and cytochrome enzymes results in an increase in the rate of photosynthesis, and a promotion in carbohydrate synthesis and accumulation

**Table 2.** Influence of bio-inoculants and irrigation intervals on the chemical analysis of *D. regia* during 2013 and 2014.

| Treatment (T) | Irrigation intervals (I) | Chemical analysis | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total chlorophylls (a+b) content (mg/g fresh matter) | | Carotenoids content (mg/g fresh matter) | | Total carbohydrates (% of dry matter) | | Proline content (µ moles/g fresh matter) | | N (% dry matter) | | P (% dry matter) | | K (% dry matter) | |
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| **Control** | | 1.15 | 1.09 | 0.55 | 0.52 | 9.00 | 7.00 | 11.00 | 10.00 | 1.49 | 1.40 | 0.23 | 0.20 | 1.40 | 1.37 |
| Full NPK | | 1.20 | 1.18 | 0.58 | 0.59 | 11.00 | 8.00 | 13.00 | 11.00 | 1.60 | 1.58 | 0.27 | 0.25 | 1.41 | 1.39 |
| AMF | | 1.65 | 1.49 | 0.81 | 0.73 | 21.00 | 17.00 | 25.00 | 21.00 | 2.49 | 2.41 | 0.49 | 0.43 | 2.31 | 2.09 |
| *A. chroococcum* | **3 Days** | 1.58 | 1.45 | 0.78 | 0.70 | 18.00 | 12.00 | 20.00 | 17.00 | 2.33 | 2.31 | 0.35 | 0.33 | 2.00 | 1.88 |
| *R. mucilaginosa* | | 1.43 | 1.37 | 0.69 | 0.65 | 15.00 | 10.00 | 17.00 | 15.00 | 1.90 | 1.85 | 0.28 | 0.27 | 1.55 | 1.52 |
| *S. cerevisiae* | | 1.60 | 1.46 | 0.80 | 0.72 | 20.00 | 14.00 | 23.00 | 19.00 | 2.46 | 2.36 | 0.39 | 0.38 | 2.10 | 1.95 |
| Mixture of all | | 1.68 | 1.53 | 0.83 | 0.77 | 23.00 | 19.00 | 28.00 | 23.00 | 2.66 | 2.56 | 0.50 | 0.47 | 2.44 | 2.17 |
| **Control** | | 0.97 | 0.85 | 0.46 | 0.41 | 12.00 | 8.00 | 15.00 | 11.00 | 1.42 | 1.36 | 0.19 | 0.16 | 1.38 | 1.33 |
| Full NPK | | 1.05 | 0.99 | 0.51 | 0.49 | 13.00 | 10.00 | 18.00 | 15.00 | 1.58 | 1.55 | 0.25 | 0.23 | 1.40 | 1.36 |
| AMF | | 1.50 | 1.35 | 0.74 | 0.65 | 24.00 | 20.00 | 31.00 | 26.00 | 2.34 | 2.34 | 0.35 | 0.34 | 1.70 | 1.64 |
| *A. chroococcum* | **6 Days** | 1.46 | 1.30 | 0.73 | 0.63 | 19.00 | 17.00. | 26.00 | 23.00 | 2.27 | 2.18 | 0.29 | 0.28 | 1.63 | 1.60 |
| *R. mucilaginosa* | | 1.35 | 1.28 | 0.65 | 0.61 | 15.00 | 12.00 | 21.00 | 17.00 | 1.82 | 1.77 | 0.23 | 0.21 | 1.31 | 1.29 |
| *S. cerevisiae* | | 1.49 | 1.33 | 0.72 | 0.65 | 21.00 | 19.00 | 30.00 | 24.00 | 2.30 | 2.26 | 0.33 | 0.31 | 1.66 | 1.61 |
| Mixture of all | | 1.54 | 1.36 | 0.77 | 0.68 | 27.00 | 23.00 | 33.00 | 29.00 | 2.57 | 2.51 | 0.40 | 0.38 | 1.75 | 1.70 |
| **Control** | | 0.83 | 0.70 | 0.38 | 0.33 | 15.00 | 13.00 | 22.00 | 16.00 | 1.34 | 1.31 | 0.14 | 0.11 | 1.27 | 1.25 |
| Full NPK | | 0.89 | 0.73 | 0.43 | 0.35 | 18.00 | 15.00 | 29.00 | 25.00 | 1.44 | 1.37 | 0.20 | 0.16 | 1.35 | 1.27 |
| AMF | | 1.34 | 1.19 | 0.66 | 0.57 | 32.00 | 25.00 | 44.00 | 43.00 | 2.18 | 2.10 | 0.28 | 0.27 | 1.53 | 1.45 |
| *A. chroococcum* | **12 Days** | 1.28 | 1.15 | 0.64 | 0.56 | 27.00 | 21.00 | 40.00 | 38.00 | 2.00 | 1.94 | 0.25 | 0.23 | 1.49 | 1.33 |
| *R. mucilaginosa* | | 1.18 | 1.10 | 0.58 | 0.53 | 21.00 | 17.00 | 36.00 | 31.00 | 1.64 | 1.58 | 0.22 | 0.21 | 1.38 | 1.31 |
| *S. cerevisiae* | | 1.31 | 1.17 | 0.65 | 0.56 | 31.00 | 23.00 | 41.00 | 40.00 | 2.17 | 2.07 | 0.27 | 0.25 | 1.51 | 1.42 |
| Mixture of all | | 1.39 | 1.20 | 0.68 | 0.59 | 34.00 | 29.00 | 49.00 | 45.00 | 2.21 | 2.16 | 0.31 | 0.29 | 1.59 | 1.47 |
| **LSD (0.05)** | | | | | | | | | | | | | | | |
| **I** | | 0.18 | 0.03 | 0.09 | 0.01 | 6.99 | 5.94 | 5.84 | 6.07 | 0.54 | 0.94 | 0.05 | 0.06 | 0.03 | 0.05 |
| **T** | | 0.16 | 0.19 | 0.08 | 0.09 | 5.96 | 3.96 | 8.52 | 6.28 | 0.98 | 0.93 | 0.07 | 0.06 | 0.33 | 0.09 |
| **I X T** | | 0.28 | 0.33 | 0.14 | 0.16 | 10.32 | 6.85 | 14.75 | 10.87 | 1.69 | 1.62 | 0.12 | 0.11 | 0.57 | 0.15 |

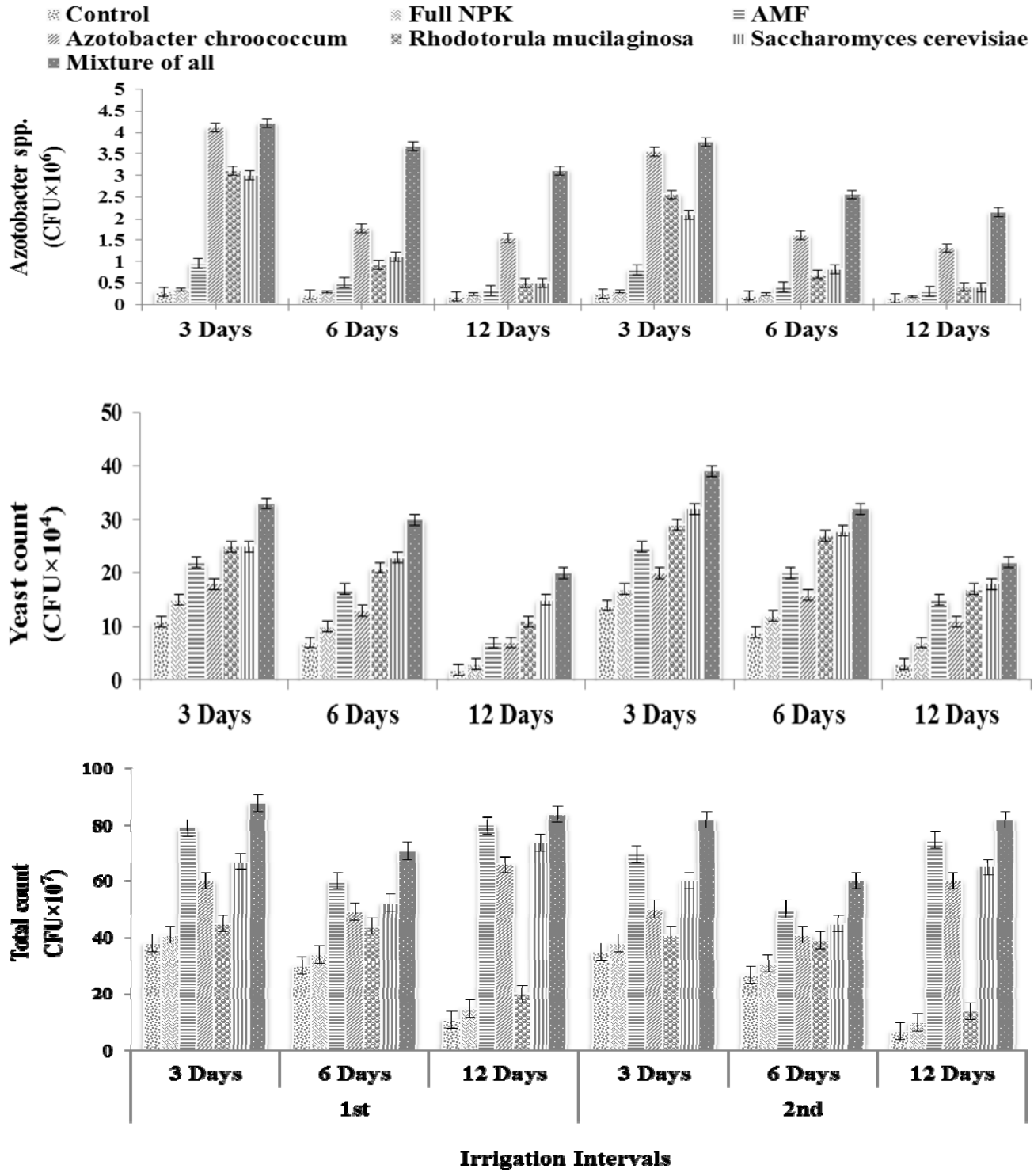


**Figure 1.** Effect of biofertilization and irrigation intervals on microbial population in the rhizosphere of *D. regia*during 2013 and 2014.

(Devlin, 1975). The obtained results are in agreement with the findings of Khalid (2006) on *Ocimum americanum* and *O. basilicum* who found that the total carbohydrates increased when the plants were inoculated with myvorrhizal fungi under water stress.

***N, P and K (%)***

The results in Table 2 also showed that the N, P and K percentages decreased steadily with prolonged irrigation intervals. Accordingly, the lowest percentages of the

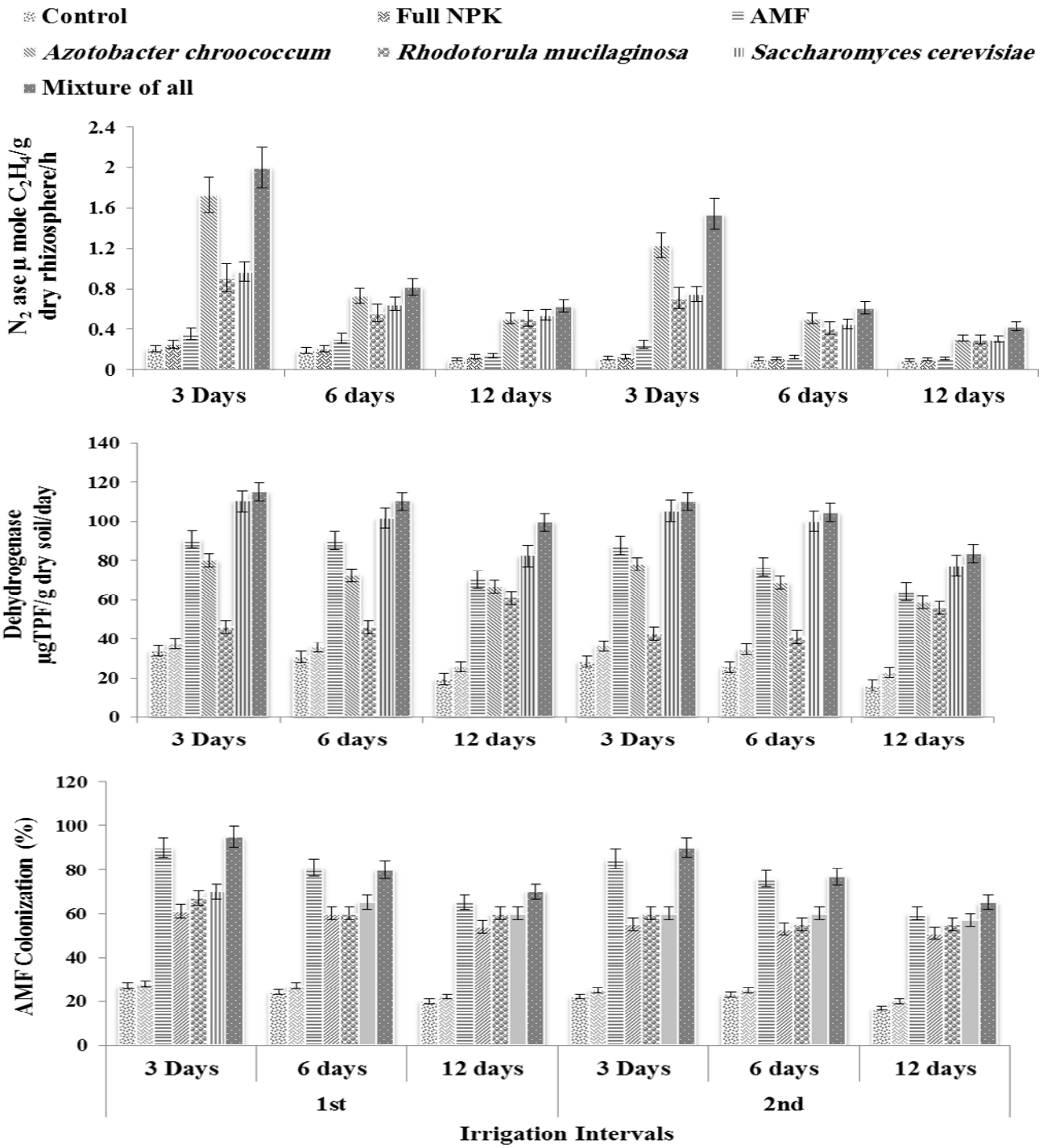


**Figure 2.** Effect of biofertilization and irrigation intervals on mycorrhizal colonization (%), dehydrogenase and nitrogenase enzyme activities during 2013 and 2014.

three nutrients were found in plants irrigated every 12 days; whereas the highest percentages were found in plants irrigated after 3 days, regardless of the effect of inoculation treatments. These results are in agreement with the findings of Jaleel et al. (2008) who indicated that water stress reduces growth by affecting various physiological and biochemical processes, such as ions uptake, translocation, and nutrient metabolism.

The results presented in Table 2 revealed that, in both seasons, *D. regia* plants inoculated with the dual bio-fertilizers had the highest N, P and K percentages in their leaf tissues, compared to un-inoculated (control) plants.

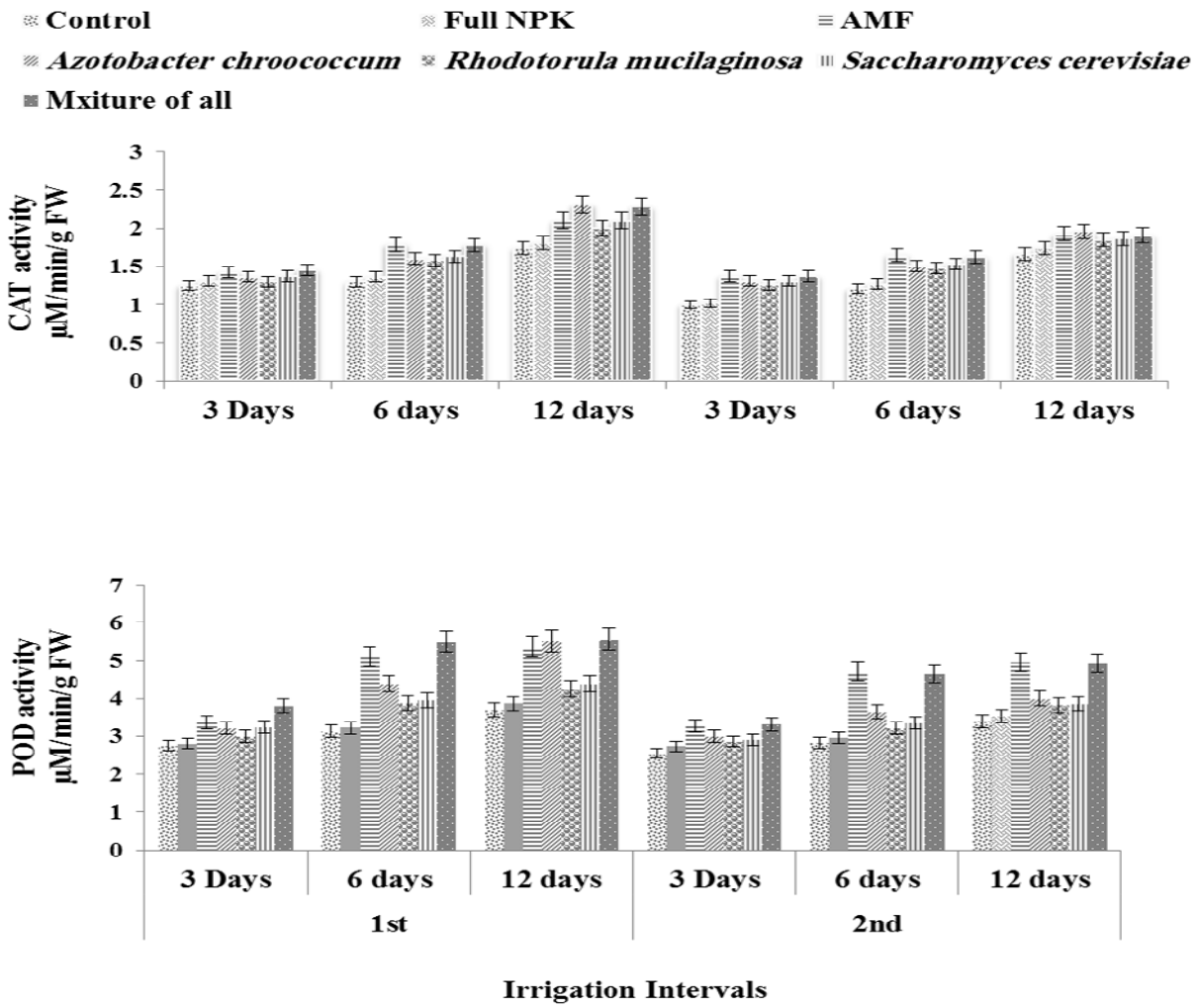


**Figure 3.** Effect of biofertilization and irrigation intervals on the activities of antioxidant enzymes (CAT and POD) of *D. regia* during 2013 and 2014.

These augmentations with reference to main macro-elements N, P and K were 55.36, 44.33 and 60.09%, respectively, in control plants. These results are in agreement with the findings of Jayakumar and Tan (2006) who indicated that seedlings of *Acacia mangium* inoculated with different strains of *Bradyrhizobium* had higher P contents compared to un-inoculated seedlings. AM fungi interact with other soil microbes like free-living nitrogen fixers and phosphate solubilizes to improve their efficiency for the biochemical cycling of elements to the host plants. Also, PGPR strains convert unavailable minerals and organic compounds into forms available to plants. In addition, PGPR strains usually have been found to increase the root length and root biomass and better developed root system. This may increase the mineral uptake in plants. This process increases nutrient uptake and availability of nutrients in the rhizosphere, resulting in an increase in plant growth and yield, as reported by Siddiqui and Mahmood (1999), Gupta et al. (2002) and Khalid et al. (2004).

**Activities of antioxidant enzymes**

Our findings showed that by increasing the irrigation intervals (12 days), the activities of catalase (CAT) and peroxidase (POD) enzymes are significantly increased (1.74 and 3.70 µM/min/g FW, respectively, in the first season;1.66 and 3.39 µM/min/g FW, respectively, in the second season) compared to the respective 3 days (1.25 and 2.75 µM/min/g FW, respectively, in the first season; 1.00 and 2.55 µM/min/g FW, respectively, in the second season) (Figure 3). Abiotic stress such as drought causes damage directly or indirectly to plants, through re-active oxygen species (ROS) formation, which increases by increase in the severity of drought conditions. This leads to the increase of tolerance to oxidative stress (Farooq et al., 2009). The increase in the activity of CAT

and POD enzymes is in agreement with those found in *Helianthus annuus* (Nazarlil et al., 2011) and *Boehmeria nitea* (Huang et al., 2013), subjected to different watering regimes.

In this study, dual bio-fertilizers led to a significant increase in the CAT and POD enzymes compared to un-inoculated (control) plants (Figure 3). Borde et al. (2012); Heidari and Golpayegani (2012) and Morteza et al. (2013) concluded that bio-fertilization can prevent oxidetive stress by increasing activities of antioxidant enzymes during periods with intense photosynthesis; elevated activity could be correlated with increased stress tolerance. Therefore, application of dual bio-fertilizers can be an important tool in *D. regia* cultivation to overcome drought stress conditions and can protect plants from drought conditions. The same previously mentioned trend was observed by other authors (Ruiz-Lozano et al, 2001; Alguacil, 2003; Saravanakumar et al., 2011).

It can be recommended to inoculate soil with *A. mycorrhizal* fungi combined with *Azotobacter* spp. and yeasts for increasing the tolerance of young seedlings of *D. regia* as well as enhancing growth, nutrition status and activities of antioxidant enzymes under drought condition, besides their safety for either environment or human health.

## Conflict of Interest

The authors have not declared any conflict of interest.

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