# Physiological effect of melatonin, IAA and their precursor on quality and quantity of chickpea plants grown under sandy soil conditions

Mohamed Elsayed. El-Awadi*, Mona Gergis. Dawood,
Yasser Refaey. Abdel-Baky, Esmat Ahmed. Hassan

(*Botany Department, National Research Centre, 33 El-Tahrir St., Dokki, 12622 Giza, Egypt*)

**Abstract:** The physiological effect of melatonin, IAA and their precursor (tryptophan) as seed priming on quantity and quality of chickpea plants was investigated during two growing seasons of 2013/2014 and 2014/2015 under sandy soil conditions at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt. Data show that melatonin treatments showed the most pronounced effect on vegetative growth parameters followed by IAA and tryptophan treatments. The highest significant increase in dry weight of shoot/plant was achieved by melatonin treatments at 0.5 and 1.0 mM. Moreover, melatonin treatments at 0.25 and 0.5 mM were the most pronounced treatments caused the highest significant increase in total photosynthetic pigments by 52.18% over control. All applied treatments caused significant increases in seed yield/feddan and its attributes (number of pods and seeds/plant as well as 100 seeds weight). IAA treatment at 20 mg $L^{-1}$ and all melatonin treatments had the most positive effect on increasing seed yield and yield attributes. It was obvious that seed yield/feddan was increased by 113%, 50.6%, 117.6% and 49.6% under the use of 20 mg $L^{-1}$ IAA, 0.25, 0.50 and 1.0 mM melatonin respectively over control. All applied treatments caused a significant increase in the nutritional values of the yielded seed (carbohydrate, oil, phenolic compound and antioxidant activity). Melatonin treatment at 0.5 mM was the most pronounced treatment, since it caused a significant increase in oil content by 44.94%, carbohydrate content by 8.12%, phenolic content by 57.14% and antioxidant activity by 9.41% over control. The results indicate that oil biosynthesis in chickpea seed responded to melatonin treatment more effectively than carbohydrate biosynthesis. Data show that application of tryptophan led to significant increases in the antioxidant activity (as DPPH- radical scavenging capacity) of the yielded seeds as compared with control plant. It is worthy to mention that melatonin treatments were the most pronounced treatments on the quality and quantity of chickpea plant grown under sandy soil conditions.

**Keywords:** Melatonin, IAA, tryptophan, chickpea, sandy soil

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## 1 Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important legumes cultivated in arid and semiarid regions of the world. Besides being an important source of human and animal food, the crop plays an important role in the maintenance of soil fertility, particularly in arid regions. Chickpea seed contains 13% to 33% protein, 40% to 55% carbohydrate and 4% to 10% oil (Stallknecht et al., 2006).Chickpeas meet adult human requirements for all essential amino acids except methionine and cysteine and have a low level of tryptophan (Abbas et al., 2013).

Seed priming is simple, cheap and safe for the environment. This technique is one of the physiological methods that allow some changes in physiological and biochemical processes before germination (Jaskani et al., 2006). Seed priming can be combined with the

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*** Corresponding author: Mohamed Elsayed. El-Awadi,** Professor of Botany Department, Research Department, National Research Centre. Email: monagergis@yahoo.com. Tel: (+202) 33371362, Fax: (+202)33370931c.

application of plant hormones (Tiryaki and Keles, 2012); biostimulators (*e.g.*, proline) (Posmyk and Janas, 2007) and melatonin(Posmyk et al., 2008; Janas and Posmyk, 2013; Dawood and El-Awadi, 2015).

L-tryptophan is an amazing amino acid. It may act as an osmolyte, ion transport regulator, modulates stomatal opening and detoxify harmful effects of heavy metals (Rai, 2002) as well as plays a defensive role in plants (Hussein et al., 2014). In addition, tryptophan improved many physiological processes such as regulation of plant growth, differentiation and metabolism of plants and increasing physiological availability of water and nutrients (El-Bassiouny, 2005; Talaat et al., 2005). Exogenous application of L-Tryptophan has been reported to improve the growth and yield of various crops (Abbas et al., 2013; Dawood and Sadak, 2007; El-Awadi and Hassan, 2010) under normal conditions or even under stressed conditions. Bakry et al. (2016) mentioned that foliar application of tryptophan under water deficit (skipping irrigation) enhanced the growth by stimulating growth regulators level (IAA) and protecting the photosynthetic apparatus. L-Tryptophan is known as a common physiological precursor of auxins (IAA) and melatonin in higher plants (Khalid et al., 2006; Chen et al., 2009).

Auxins are phytohormones involved in mediating a number of essential plant growth and developmental processes, such as cell elongation and division, induction of root growth as well as flower and fruit development. It is known that the range between beneficial and toxic effects of auxin can be quite narrow (Salisbury and Ross, 1992). Generally auxin is reported to increase plant growth when applied at low concentrations and inhibit growth at higher concentrations (Gaspar et al., 2002; Hussain et al., 2010). The application of these hormones at low concentration regulates growth, differentiation and development, either by promotion or inhibition and allows physiological processes to occur at a normal rate (Naeem et al., 2004; Vwioko and Longe, 2009).

IAA is considered the most important native auxin in higher plants (Khalid et al., 2004). It stimulates root elongation and lateral root production (Lippmann et al., 1995), regulates the physiological process through the enzymatic reactions and the nucleic acid synthesis (Naguib et al., 2003).

The chemical structure of melatonin (indoleamine) is similar to auxin-IAA hormone.

Melatonin (N-acetyl-5-methoxytryptamine) (MEL) can act as a potential modulator of plant growth and development in a dose-dependent manner (Hernandez-Ruiz and Arnao, 2006; Li et al., 2012). In plants, MEL is considered to be involved in the regulation of circadian rhythm (Tal et al., 2011) as well as in many physiological processes, *e.g.* root and shoot development (Park, 2011), flowering, flower and fruit development or delaying leaf senescence(Kola´r et al., 2003; Wang et al., 2012; Park et al., 2013) ion homeostasis (Sarropoulou et al., 2012). Due to the fact that, melatonin possesses both lipophilic and hydrophilic properties, it may be easy for the molecule to cross morpho-and physiological barriers with minimal difficulty, resulting in the rapid transport of the molecule into plant cells. In recent years, a number of studies have recognized the dual role of melatonin in plants as a protector against abiotic and biotic stresses (Li et al., 2012; Wang et al., 2012; Manchester et al., 2000). Melatonin as an antioxidant in plants and proven a potent free-radical scavenger (Tan et al., 2002). These findings support the concept that one important role of melatonin in plants may be to protect the plant from any stressful condition and to prevent injuries induced by oxidative stress at the cellular level (Tan et al., 2002; Hardeland et al., 2001).

Melatonin was also reported delaying senescence of leaves, both in monocots and dicots (Fletcher and Sopher, 1997). Similar to IAA, melatonin may stimulate growth in etiolated lupine (*Lupinus albus*) and coleoptiles of canary grass (*Phalaris canariensis*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and oats (*Avena sativa*), with a relative auxinic activity of 10%-55%. Arnao and Herna´ndez-Ruiz (2006) hypothesized that exogenous melatonin might cause changes in the concentration of endogenous free IAA. Incubating *Hordeum vulgare* L. (barley) leaves with increasing concentrations of exogenous melatonin in the growing medium resulted in a dose-dependent accumulation of the indole in the leaves (Arnao and Hernandez-Ruiz, 2008).

This work was conducted to explore the physiological effect of melatonin, IAA and their precursor as seed priming on quantity and quality of chickpea plants grown under sandy soil conditions.

## 2 Materials and methods

### 2.1 Experimental procedure

Two field experiments were carried out at the Experimental Station of the National Research Centre (Research and Production Station, Nubaria region, Behira Governorate, Egypt) during two successive seasons of 2013/2014 and 2014/2015.

Chickpea (cv Giza 195) seeds were sourced from Agriculture Research Centre, Egypt, cleaned and soaked with tryptophan or IAA solutions at 10, 20 and 30 mg $L^{-1}$ or in melatonin at 0.25, 0.50 and 1.0 mM for 12 hours and left to dry in open air. Chickpea seeds were sown on 18th November in the two seasons. The experimental design was randomized complete block design with three replications. Each replicate included ten plots and the plot area was 10.5 $m^2$ (1/400 of feddan), three meters in length and three and half meters in width. Each plot contained five ridges. Seeds were planted on two sides of the ridge in the hills and hills were spaced at 20 cm distance, and three seeds were sown in each hill. The plants were thinned to one plant per hill at 21 DAS. Soil preparation, fertilizer application and cultural operations followed the normal practices of chickpea cultivation in the vicinity.

### 2.2 Data recorded

A random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment to study the morphological characters at the age of 60 days after sowing.

Vegetative growth characters (shoot height (cm), number of branches/plant, fresh and dry weight of shoots ($gplant^{-1}$) were recorded during the vegetative stage. Photosynthetic pigments (chlorophyll a,b and carotenoids) were determined in fresh leaves. While, soluble protein was determined in dried leaves. At harvest time, a random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment to determine the mean values of yield and its related parameters, i.e., Number of pods and seeds per plant; weight of 100 seeds (g); seed yield (Kg/Feddan).Carbohydrates and oil percentage as well as phenolic content and antioxidant activity were determined in the yielded seeds.

### 2.3 Biochemical Studies

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the fresh leaves at 60 days from sowing were determined as the method described by Moran (1982). Soluble protein was determined according to Bradford (1976). The oil content of the yielded seeds was determined according to the procedure reported by A.O.A.C. (1990).Total carbohydrate was determined according to Dubois et al. (1956). Total phenolic content was measured as the method described by Zhang and Wang (2001).The free radical scavenging activity was determined according to Brand-Williams et al. (1980) using the 1.1-diphenyl-2- picrylhydrazyl (DPPH) reagent.

*Statistical Analysis*

Average of two seasons was statistically analyzed as randomize complete block design according to Snedecor and Cochran, (1980). The Duncan multiple range test was used to compare the treatments means (Duncan, 1955). The MSTATC (1989) program was used in this connection.

## 3 Results and discussion

### 3.1 Growth parameters

Regarding vegetative growth parameters, fresh and dry weight of shoot/plant were significantly increased by all applied treatments as shown in Table 1, whereas shoot height and number of branches/plant were significantly increased by most treatments. Melatonin treatments showed the most pronounced effect on vegetative growth parameters followed by IAA and tryptophan treatments. The highest significant increase in dry weight of shoot/plant was achieved by melatonin treatments at 0.50 and 1.0 mM.

Application of L-tryptophan has proved very fruitful for increasing growth of many crops like chickpea (Abbas et al., 2013); wheat (Mohite, 2013); quinoa (Bakry et al., 2016) and snap bean plants (El-Awadi et al., 2011).

Auxin promotes cell elongation by causing acidification of cell walls, which increases their plasticity, and walls expand due to the force of cell internal turgor pressure. Auxin is involved in mitotic activity in sub-apical tissues, resulting in increased plant growth (Abel and Theologis, 2010). In addition, Mohite (2013) mentioned that the readily available tryptophan in treated plants is converted into IAA that improves plant growth.

It is worth to mention that IAA involved in different functions dealing with growth as it controls vascular tissue development, cell elongation, and apical dominance (Wang et al., 2001); enhances cell division (Chaudhry and Khan, 2007); increases cell expansion, stem elongation accompanied by expansion in shoot diameter (Arif et al., 2001; Rautela et al., 2001).

Melatonin has similar chemical structure as auxin-IAA so, it may play a similar function as an auxin and mediate many physiological processes in plants i.e. growth regulation and ion homeostasis and increase vegetative growth in a number of plant species under normal conditions or even under stressed conditions (Hernandez-Ruiz and Arnao, 2008; Kola´r and Macha´ckova, 2005).

**Table 1 Effect of IAA, tryptophan and melatonin on some vegetative growth parameters of chickpea plant**

| Treatments | | Shoot height, cm | Branches number/ plant | Fresh shoot weight/ plant, g | Dry shoot weight/ plant, g |
|---|---|---|---|---|---|
| Control | | 28.67f | 1.5c | 6.17h | 1.24f |
| IAA | 10 mg L$^{-1}$ | 32.00de | 1.67bc | 7.33g | 1.61e |
| | 20 mg L$^{-1}$ | 34.67bc | 2.83a | 9.70de | 2.57c |
| | 30 mg L$^{-1}$ | 34.67 bc | 1.83 bc | 8.60 ef | 2.25 cd |
| Tryptophan | 10mg L$^{-1}$ | 30.00ef | 1.5c | 7.70fg | 1.78e |
| | 20 mg L$^{-1}$ | 33.33cd | 2.5ab | 8.93de | 1.85e |
| | 30 mg L$^{-1}$ | 31.33de | 2.5ab | 9.97cd | 1.93de |
| Melatonin | 0.25 mM | 36.33ab | 2.33abc | 11.00bc | 2.61c |
| | 0.50 mM | 38.67a | 3.00a | 15.00a | 4.66a |
| | 1.0 mM | 37.00ab | 2.5ab | 12.03b | 3.36b |
| L.S.D. at 5% | | 2.40 | 0.8 | 1.14 | 0.36 |

### 3.2 Photosynthetic pigments

Chlorophyll a,b and carotenoids were increased under the effect of IAA, tryptophan and melatonin at all concentrations as shown in Table 2. Melatonin treatments at 0.25 and 0.5 mM were the most pronounced treatments and caused the highest significant increase in total photosynthetic pigments by 52.18% over control. The positive effect of amino acids application on the photosynthetic pigments might be attributed to either the preservation of chromoproteins or activation the formation of the enzymes required for building of chlorophyll or carotenes (Hall and Rao, 1987). In this connection, Barazan and Friedman (Barazanand Friedman, 2000) concluded that tryptophan induced affect the chloroplast biosynthesis through its role in IAA biosynthesis. Earlier, Jacobs (1979) found that, presumably IAA acts as aco-enzyme in the metabolism in higher plants, thus it plays an important role in the formation of the photosynthetic pigments.

The promotive effect of melatonin on photosynthetic pigments might be due to its role in improving the ultrastructure of chloroplasts, or due to raising the antioxidant enzyme activities, antioxidant contents and thus inhibiting the production of reactive oxygen species and thereby delay the senescence process (Arnao and Herna´ndez-Ruiz, 2006). Melatonin molecule significantly reduced chlorophyll degradation, suppressed the up-regulation of senescence-associated gene, and increased the photosynthetic efficiency of many plants (Wang et al., 2012, Tan et al., 2012).

### 3.3 Soluble protein

Table 2 shows that all treatments caused marked increases in soluble protein. IAA at 30ppm was the most pronounced treatment followed by 10 ppm tryptophan and 1.0 mM melatonin. Application of tryptophan increased free amino acids content in the snap bean leaves (El-Awadi et al., 2011). Earlier, Ahmed et al. (2001) mentioned that the amino acids increment due to spraying with IAA would be attributed to either the increase in the rate of the amino acid synthesis or to the effect of these compounds on the enzymatic system which stimulates the utilization of carbohydrates for amino acids synthesis. Sallam (Sallam, 1993) indicated that the externally addition of IAA could be stimulate the synthesis of newly protein and RNA in guar leaves.

### 3.4 Seed yield and yield attributes

Table 3 shows that all applied treatments caused significant increases in seed yield/faddan and its attributes (number of pods and seeds/plant as well as 100 seeds weight) except IAA treatment at 10 mg L$^{-1}$ caused non-significant increase in seed yield/feddan and

100 seed weight. IAA treatment at 20 mg $L^{-1}$ and all melatonin treatments had the most positive effect on increasing seed yield and yield attributes. It was obvious that seed yield/feddan was increased by 50.6%, 117.6% and 49.6% over control by the effect of 20 mg $L^{-1}$ IAA, 0.25, 0.50 and 1.0 mM melatonin treatment respectively.

**Table 2 Effect of IAA, tryptophan and melatonin on photosynthetic pigments (mg $g^{-1}$ fresh weight) and soluble protein (g 100 $g^{-1}$ dry weight) of chickpea leaf tissues**

| Treatments | | Chlorophyll a | Chlorophyll b | Carotenoid | Total photosynthetic pigments | Soluble protein |
|---|---|---|---|---|---|---|
| Control | | 1.551d | 0.514d | 0.340abc | 2.405c | 3.99f |
| IAA | 10 mg $L^{-1}$ | 1.791bcd | 0.848bcd | 0.365abc | 3.003 abc | 4.44bcde |
| | 20 mg $L^{-1}$ | 1.856abc | 0.916bcd | 0.344abc | 3.116 abc | 4.48 bcd |
| | 30 mg $L^{-1}$ | 1.834abcd | 0.923bcd | 0.300 c | 3.056 abc | 4.25 de |
| Tryptophan | 10 mg $L^{-1}$ | 1.709 cd | 0.827bcd | 0.325bc | 2.861 bc | 4.69 ab |
| | 20 mg $L^{-1}$ | 1.679 d | 0.679 cd | 0.424 a | 2.781 bc | 4.61abc |
| | 30 mg $L^{-1}$ | 2.065 abc | 1.059abcd | 0.336abc | 3.459 ab | 4.81 a |
| Melatonin | 0.25 mM | 2.051 abc | 1.318ab | 0.297 c | 3.666 a | 4.19ef |
| | 0.50 mM | 2.120 ab | 1.228 abc | 0.327bc | 3.675 a | 4.34 de |
| | 1.0 mM | 2.159 a | 1.551 a | 0.400 ab | 4.110 a | 4.40 cde |
| L.S.D. at 5% | | 0.326 | 0.5 | 0.094 | 0.732 | 0.24 |

**Table 3 Effect of IAA, tryptophan and melatonin on seed yield and yield attributes of chickpea plant**

| Treatments | | Number of pods/plant | Number of Seeds/plant | Seed yield/ feddan, kg | 100 seeds weight, g |
|---|---|---|---|---|---|
| Control | | 7.00g | 9.500g | 648.8c | 23.80e |
| IAA | 10 mg $L^{-1}$ | 10.00f | 11.80f | 676.6c | 25.00e |
| | 20 mg $L^{-1}$ | 26.20b | 33.30b | 1382.0a | 28.20d |
| | 30 mg $L^{-1}$ | 16.30d | 20.70d | 686.2c | 20.10f |
| Tryptophan | 10 mg $L^{-1}$ | 12.40e | 17.40e | 658.3c | 25.10e |
| | 20 mg $L^{-1}$ | 18.50c | 21.50d | 667.0c | 28.30d |
| | 30 mg $L^{-1}$ | 11.80e | 12.40f | 670.0c | 30.30c |
| Melatonin | 0.25 mM | 27.50b | 28.50c | 977.3b | 34.20b |
| | 0.50 mM | 30.30a | 37.50a | 1412.0a | 36.20a |
| | 1.0 mM | 26.50b | 28.40c | 970.3b | 31.80c |
| L.S.D. at 5% | | 1.72 | 2.16 | 98.69 | 1.61 |

The increase in plant biomass and yield due to tryptophan treatments may be attributed to the positive influence of L-TRP on the cellular division; the production of phytohormones, e.g., auxin (Zahir et al., 2010; Abbas et al., 2013) and enhancing nutrients uptake and assimilation (Coruzzi and Last, 2000) as well as enhancing protein synthesis and delaying senescence (El–Bassiouny et al., 2005). Moreover, the increase in seed weight could be attributed to the promotive effect of tryptophan in increasing the amount of assimilates as well as increasing their translocations from the leaves to the fruits and hence increase seed weight (Dawood and Sadak, 2007).

Regarding IAA influence, it was note that the enhancement in plant growth by IAA application may be attribute to its role in enlarging leaves and increasing photosynthetic activities in plants (Naeem et al., 2004) and activating the translocation of carbohydrates during their synthesis (Awan et al., 1999) thus leading to increase crop yield. Auxin application increased pea and wheat growth and yield based on varietals sensitivity and correct application timing (Hussain et al., 2011).

Melatonin may mediate many physiological processes in plants i.e. growth regulation and ion homeostasis and increase vegetative growth in a number of plant species leading to increments in seed yield (Janas and Posmyk 2013; Hernandez-Ruiz and Arnao, 2006; Li et al., 2012; Sarropoulou et al., 2012; Kola´r and Macha´ckova, 2005).

### 3.5 Nutritive value of the yielded seeds

All applied treatments caused significant increase in the nutritional values of the yielded seed (carbohydrate, oil, phenolic compound and antioxidant activity) as shown in Table 4. It was noted that melatonin treatments had the highest positive effect on the nutritive value of chickpea seeds followed by IAA and tryptophan treatments. Melatonin treatment at 0.5 mM was the most pronounced treatment, since it caused significant increase in oil content by 44.94%, carbohydrate content by 8.12%, phenolic content by 57.14% and antioxidant activity by 9.41% over control. These results indicate that oil biosynthesis in chickpea seed responded to melatonin treatment more effectively than carbohydrate biosynthesis.

**Table 4 Effect of IAA, tryptophan and melatonin on nutritive value (%) of the yielded chickpea seeds**

| Treatments | | Oil content | Total carbohydrate | Phenolic content | Antioxidant activity |
|---|---|---|---|---|---|
| Control | | 4.25h | 58.19f | 4.34e | 54.20d |
| IAA | 10 mg $L^{-1}$ | 5.01fg | 59.09de | 5.45cd | 57.34bc |
| | 20 mg $L^{-1}$ | 5.07ef | 62.11ab | 5.22d | 57.78abc |
| | 30 mg $L^{-1}$ | 5.34de | 59.67cd | 5.67c | 57.13c |
| Tryptophan | 10 mg $L^{-1}$ | 4.78g | 58.69ef | 5.28d | 57.93abc |
| | 20 mg $L^{-1}$ | 5.22def | 60.37c | 5.74c | 57.81abc |
| | 30 mg $L^{-1}$ | 5.46cd | 61.84b | 5.73c | 57.34bc |
| Melatonin | 0.25 mM | 5.72bc | 61.59b | 5.20d | 58.83ab |
| | 0.50 mM | 6.16a | 62.92a | 6.82a | 59.30a |
| | 1.0 mM | 5.85b | 61.89b | 6.17b | 58.87ab |
| L.S.D. at 5% | | 0.27 | 0.85 | 0.27 | 1.37 |

El-Bassiouny (2005), Abdel-Monem et al. (2010) found that, total carbohydrate was increased in wheat and sunflower plants respectively due to tryptophan application. Moreover, the promotive effect of the amino acids on the total carbohydrates content may be due to their important role on the biosynthesis of chlorophyll molecules, which in turn affected carbohydrate content. IAA also activates sugar translocation during the carbohydrates synthesis, (Akhtar et al., 2012). Altman and Wareing (1975) suggested that, there is a close relationship between the enhancement of sugar accumulation and IAA treatments.

Regarding oil yield, Farooqui et al. (2005) reported that indole acetic acid (IAA) application increased oil yield enormously in *Cymbopogon martinii* and *Cymbopogon winterianus.* Faizanullah et al. (2010) reported that judicious application of growth hormone increases seed yield in linseed and consequently oil yield.

The enhancement effect of melatonin on photosynthetic pigments (Table 2) might interpret the overproduction of total carbohydrates, and concomitant with the enhancement of quality and quantity of yield. Melatonin is seen to be an agent with auxinic activity similar to IAA (Afreen et al., 2006; Arnao and Herna´ndez-Ruiz, 2006; Arnao and Hernandez-Ruiz, 2007; Posmyk et al., 2008; Chen et al., 2009).

### 3.6 Regarding phenolic content and antioxidant activity

The increase in total phenolic compounds (Table 4) may inhibit IAA oxidase activity and leading to auxin accumulation and reflected in stimulating the growth and yield of plant as reported by Dawood and Sadak (2007). Naguib et al. (2003) mentioned that total phenolic compounds of the periwinkle plants were significantly increased due to the application of IAA (50 or 100 ppm) compared with control plants. In addition, melatonin treatment increased the synthesis of phenolic content as reported by Zhang et al. (2015). Szafranska et al. (2012) showed that melatonin added to *Vigna radiate* L. Seeds protected the roots of chilled seedlings after re-warming and increased the synthesis of phenolic compounds. Other researchers stated that the increase in total phenolic lead to increases in antioxidant activity (Zilic et al., 2011).

Data in (Table 4) show that application of tryptophan led to significant increases in the antioxidant activity (as DPPH-radical scavenging capacity) of the yielded seeds as compared with control plant. The observed increase in antioxidant activities may be attributed to the positive role of plant growth regulator on detoxification of reactive oxygen species (Faize and Burgos, 2011). The reducing power of MEL explains some aspects of antioxidant activity and can be directly related to the amount of phenolic (Siddhuraju et al., 2002).

Paredes et al. (2009) indicated that elevated melatonin may confer higher antioxidant protection in plants especially in environments where free radicals cannot be detoxified enzymatically, as in the case of dry seeds (Hardeland et al., 2007).

It is worthy to mention that melatonin treatments were the most pronounced treatments on the quality and quantity of chickpea plant grown under sandy soil conditions. The pronounced effect of melatonin may be attributed to its role as strong antioxidant that can cross-physiological barriers to prevent oxidative damage in both lipid and aqueous environments due to its amphiphilic structure (Reiter et al., 2004). Antioxidant activity of MEL seems to participate in: (i) the direct scavenging of free radicals, (ii) stimulation of antioxidant enzymes, (iii) augmentation of the activities of other antioxidants (iv) protection of antioxidant enzymes against oxidative damage, or (v) an increase in the efficiency of the mitochondrial electron transport chain, thereby lowering electron leakage and the generation of free radicals (Wang et al., 2012). Moreover, beneficial effects of melatonin may also result from its signaling function, through the induction of different metabolic pathways and stimulate the production of various substances (Barazan and Friedman, 2000).

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