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Assessment of the Responses of Chickpea (*Cicer arietinum* L.) to the Exogenous Application of Gibberellic Acid and Indole Butyric Acid at Different Crop Development Stages

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops in the world. In Tunisia, the chickpea is vulnerable to fluctuation in production in the last few decades. However, phytohormones are known to play crucial roles in regulating different development processes in plants.

Methods: This research was conducted in Petri dish and pot experiments in order to determine the effect of gibberellic acid (GA3) and indole butyric acid (IBA) phytohormones on some growth parameters of chickpea. In this study, GA3 at concentrations of 0, 0.05, 0.1, 0.15 and 0.2 g/l and IBA at concentrations of 0, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} g/l were applied at different stages of chickpea development.

Result: Results of Petri dishes trial revealed that IBA (10^{-4}g/l) and GA3 (0.2 g/l) hormones improved significantly the shoot and root length of chickpea plant. Results of pots pointed out that the chickpea achieves the highest plant height when IBA (10^{-4} or 10^{-3} g/l) and GA3 (0.2 g/l) were used at pre-flowering stage. The IBA and GA3 application with concentrations of 10^{-3} g/l and 0.1 g/l, respectively allowed the highest nodule number at post-flowering stage. The parameters number of branches, fresh and dry weight of the aerial part and leaves number were improved in chickpea plants treated with IBA and GA3 at pre-flowering and post-flowering, respectively. Similarly, the number of flowers was promoted by the two hormones. The weight and the number of seeds were significantly enhanced by all IBA treatments in plants. An increase in weight and the number of seeds was observed at post-flowering phase of GA3 treatment. Thus, these results identified the beneficial effect of tested phytohormones in chickpea growth.

Key words: Chickpea, Gibberellic acid, Growth parameters, Indole butyric acid, Phytohormones.

INTRODUCTION

Plant growth regulators also called phytohormones, are organic chemical substances produced naturally in higher plants, involving in all the factors of development and growth within plants (Han *et al*., 2018). They act at a site remote from its place of synthesis and were active in very low concentrations (Weyers and Paterson, 2001). In general, plant growth regulators include auxins, gibberellins, cytokinins, ethylene and growth inhibitors such as abscisic acid. In addition, brassinosteroids, jasmonates, salicylic acid, polyamines, nitric oxide and strigolactones are also, currently, classified as plant hormones (Hemelíková *et al*., 2021).

Previous studies revealed that auxin and gibbrellic acid are widely used to enhance growth as well as productivity in different plant species by regulating several physiological mechanisms (Tobaruela *et al*., 2021; Mahmoudi *et al*., 2022).

Auxins such as Indoleacetic acid, Phenylacetic acid, Indolebutyric acid and Naphthalene acetic acid, are a class of phytohormones that played a major role on several plant growth aspects and developmental processes, where they promote cell division, cell expansion and transition of cell from dormancy to active status facilitating plant growth (Del Pozo *et al*., 2005; Salama *et al*., 2014). They also control the root initiation, vascular tissue formation and shoot elongation (Kucera *et al*., 2005; Lau *et al*., 2009; Hopkins *et al*., 2019). In addition to development processes, compound auxins played crucial roles in the response to biotic and abiotic stresses (Egamberdieva, 2009; Singh *et al*., 2017).

Gibberellic acid (GA3) is a group of pentacyclic diterpene acid compound. It is familiar for its role in increasing seed germination, stem and root elongation, leaf expansion, flowering and fruit generation as GA3 coordinate different metabolic processes, activity of various enzymes and gene expression (Hooley, 1994; Colebrook *et al*., 2014;

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Ullah *et al*., 2018). Previous studies also suggested that GA3 is involved in relieving various environmental stresses and senescence (Miceli *et al*., 2019; Fathi-Najafabadi *et al*., 2021). Chickpea (*Cicer arietinum* L.) is an annual legume of the family *Fabaceae* and it is as self-pollinated plant with 16 chromosomes (2n=16). It is the second most vital food legumes of the world in 2021, with a global production of about 15.87 million tons after dry beans (27.72 million tons) (FAO, 2021). Chickpea is popular in many countries around the globe due its high protein (18%-24%) and carbohydrates (60%-70%) content in its grains (Ahmad *et al*., 2016; Saxena *et al*., 2023). However, its annual production has fluctuated in recent years due to the negative impact of abiotic and biotic factors. Previous researches indicated that the use of growth regulators can improve the various development aspects of chickpea (Shankar *et al*., 2020; Tiwari and Bhatia, 2020; Saral *et al*., 2021).Therefore, the aim of the present study was to evaluate the effect of two phytohormones (gibberellic acid and indole butyric acid) on growth traits of chickpea after exogenous application on different growth stages.

MATERIALS AND METHODS

Plant material

The seeds of chickpea variety (Amdoun 1) used in this work, were provided by Field Crops Laboratory of the National Institute for Agricultural Research of Tunisia (INRAT). This variety is known for its high productivity [large seed (45-48 g/ 100 seeds)] and its resistance to race 0 of *Fusarium oxysporum f. sp. ciceris*. It was released by INRAT in 1987 selected from the local population (PL-Se-Be-48) from Amdoun region.

Petri dish experiment

For germination assays, chickpea seeds were surface-sterilized for 3 min in 0.1% mercury chloride solution ($HgCl_2$) and then rinsed 5 times with sterilized distilled water. The disinfected chickpea seeds were placed on sterilized filter papers socked in 20 ml of different concentrations of growth substances (Gibberellic Acid (GA3) and Indole Butyric Acid (IBA)) in glass Petri dishes (10 cm diameter) and cultured at 25°C for 12 days, under fluorescent white light in a growth chamber.

The preparation of IBA and GA3 solutions was made from 2 commercial products: Exuberone (containing 4 g/l of (Indole Butyric Acid) and Gibrex-16 (containing 16 g/l of Gibberellic Acid), respectively. The concentrations of phytohormones used in this study are the following: IBA: 0; 10^{-5}; 10^{-4}; 10^{-3} and 10^{-2} g/l and GA3: 0; 0.05; 0.1; 0.15 and 0.2 g/l. Irrigation is carried out with distilled water every day during the trial period. The control received no phytohormone. After 3 days, the germination was over and root length (cm) and shoot length (cm) were scored four times (every 3 days).

Pot experiment

Pots were filled with soil at 2 kg pot. The soil was collected from the area of agriculture site in INRAT (National Institute for Agricultural Research of Tunisia). The soil is rich in *Rhizobium ciceri* necessary for the process of nodulation, therefore no inoculation of chickpea seeds was carried out. The physico-chemical characteristics of the soil are shown in Table 1. This study was conducted under natural conditions at INRAT during 2022.

In this experiment, six chickpea seeds were sown directly into each pot. After emergence, three plants per pot were maintained for this trial. Five hundred ml of the phytohormones (IBA AND GA3) were applied directly in the soil (root zone). The doses of growth substances used are the following: 0.1 and 0.2 g/l for GA3 and 10^{-4} and 10^{-3} g/l for IBA. These doses were selected for their positive effect on chickpea growth in Petri dishes experiment. The hormonal treatments were applied during different vegetative stages: at germination then at pre-flowering or at pre-flowering or at post-flowering. Plants were watered when necessary. The main cultural interventions focused on manual weeding to eliminate especially the dodder which appeared after a few days of sowing.

Plant were harvested at maturity and data about number of nodules, plant height, number of branches (ramifications), plant aerial part fresh and dry weights, flowers number, leaves number, seeds number and seeds weight were recorded.

Experimental design and data analysis

The trial was established according to a randomized complete design (CRD). Statistical analyses were performed using R software. Mean comparison was based on Duncan's multiple ranges classification test at P=0.05. The experiments were repeated five times.

RESULTS AND DISCUSSION

Overall, plant growth regulators cause physiological and morphological changes that are controlled by their concentrations, tissue sensitivity and species involved (Llanes *et al*., 2019). The main purpose of this study was to know whether phytohormones like indole butyric acid and gibberellins, would have any better response than untreated control on different agro-morphological parameters of chickpea.

The results of Petri dishes experiment denoted that the shoot length varied in different doses of exogenous GA3 (Table 2). The shoot length was significantly improved with all GA3 doses at 6 and 9 days after hormone application. Furthermore, the concentrations 0.1 g/l and 0.2 g/l increased significantly ($P<0.05$) the shoot length especially at 12 days after hormone treatment. The results in pots confirmed those found in Petri dishes conditions (Table 6). The different GA3 treatments increased significantly ($P<0.05$) the plant height compared with the control. Our results showed that the best GA3 treatment for highest plant height was 0.2 g/l. A previous study revealed that gibberelic acid treatment (0.2 mg/l) obtained the maximum shoot elongation of African violet (*Saintpaulia ionantha*) with an average of 19.9 mm (Ghasemi *et al*., 2012). It was also revealed that the treatment with GA3 at 20 mg/l concentration increased the cell size in higher plants of *Chlorella sorokiniana* (Ozioko *et al*., 2015). Another study indicated that the GA3 may play a role in stimulating cell elongation and expansion but not in cell division of plant (Romanenko *et al*., 2016).

Studies of GA3 signal transduction, using genetic approaches, GA3-regulated transcriptional responses are controlled by the DELLA proteins, which function by repressing different types of transcription factors (Lantzouni *et al*., 2020).

The results of Petri dishes experiment showed that IBA hormone increased significantly (P<0.05) the shoot length under 10^{-4} and 10^{-3} g/l compared to the untreated plant. In contrast, the shoot length decreased significantly (P<0.05) compared to the control after the IBA treatment with 10^{-5} and 10^{-2} g/l concentrations (Table 3). Overall, the findings of pots revealed that all IBA treatments improved the plant height. A significant (P<0.05) differences between treated and control plants were only observed in IBA treatment (10^{-4} g/l and 10^{-3} g/l) at pre-flowering stage (Table 6). This is in agreement with the results of Zayed *et al*. (2017) who reported that the plant height was significantly increased due to auxin treatments in two sunflower cultivars (Sakha 53 and China) grown under saline stress (120 mM NaCl). It has also been postulated that the application of Indole-3-acetic acid (auxin) improved the length and dry matter yield of shoot on wheat plant (Youssef *et al*., 2020). In this context, auxins may enhance plant growth by stimulating the photosynthesis by raising the contents of chlorophylls and by activating cellular redox systems (Piotrowska-Niczyporuk and Bajguz, 2014; Dao *et al*., 2018).

Table 1: The physico-chemical characteristics of the soil used in this study.

Soil components	Quantity
Clay	35%
Fine silt	21%
Coarse silt	3%
Fine sand	30%
Coarsesand	11%
Total limestone	25%
Active limestone	11%
Carbon	0.76%
Assimilable P_2O_5	19 ppm
Assimilable K_2O	299 ppm
Total nitrogen	0.9 g/kg

Regarding the effect of phytohormone on the radicle length, the results of Petri dishes experiment showed that the IBA concentrations (10^{-5} g/l and 10^{-4} g/l) increased significantly (P<0.05) the radicle length (Table 4). However, the treatment with IBA concentrations (10^{-3} and 10^{-2} g/l) reduced significantly (P<0.05) the radicle growth compared to the control plants. Wolters and Jürgens (2009) suggested that the auxin increased roots branching, which is important in enhancing drought tolerance. According to Tilahun *et al*. (2019), the tip cutting with the 11 g/l IBA treatment increased the length and number of roots of *Araucaria heterophylla* species. Chauhan *et al*. (2015) also observed that the application of IAB (0.5 mg/l) promoted the root initiation and growth in "*Allium hookeri* Thw. Enum." plant.

The results of Petri dishes experiment showed that the concentration of GA3 at 0.2 g/l increased significantly (P<0.05) the radicle length compared to the control in the different dates of the hormonal treatments (Table 5). However, for the other concentrations, a decrease in radicle length was observed.

The exogenous application of IBA was found positive and suitable in the level of nodulation. As shown in Table 6, the average number of nodules was 18.2 for the control plant. After IBA treatment, the nodules number was significantly (P<0.05) increased compared to control plant for various hormone treatment doses and different plant development stages. The highest number of nodules was recorded for the IBA treatment (10^{-3} g/l) on post-flowering.

Table 2: Variation in shoot length (cm) according to hormone treatment (GA3) from 3 to 12 days after germination in Petri dishes.

Concentrations of GA3 (g/l)	3 days	6 days	9 days	12 days
0	1.56ab±0.09	2.18a±0.25	2.64a±0.07	3.68a±0.05
0.05	1.42a±0.09	2.99b±0.09	3.34b±0.24	4.12ab±0.11
0.1	1.88b±0.09	3.18b±0.24	3.66b±0.14	4.38b±0.12
0.15	1.73ab±0.23	2.76b±0.22	3.44b±0.14	4.06ab±0.10
0.2	1.93b±0.15	3.74c±0.05	3.68b±0.06	4.38b±0.12

Average values with the same letter per column are not significantly different (P=0.05, Duncan test).

Table 3: Variation in shoot length (cm) according to hormonal treatment (IBA) from 3 to 12 days after germination in Petri dishes.

Concentrations of IBA (g/l)	3 days	6 days	9 days	12 days
0	1.56b±0.06	2.18c±0.25	2.64c±0.07	3.68c±0.05
10^{-5}	1.48b±0.07	1.7b±0.09	2.04b±0.11	2.34b±0.11
10^{-4}	2.46c±0.00	2.72d±0.00	3.5d±0.00	4.2d±0.00
10^{-3}	2.36c±0.00	2.8d±0.00	3.9e±0.00	4.3d±0.00
10^{-2}	1.08a±0.05	1.28a±0.07	1.74a±0.10	1.88a±0.12

Average values with the same letter per column are not significantly different (P= 0.05, Duncan test).

These data are in agreement with Hirsch *et al.* (1989) who showed that auxin transport inhibitors (N-1-naphthyl) phthalamic acid (NPA) and 2, 3, 5-triiodobenzoic acid (TIBA) evolved the nodules formation *in Medicago sativa*. de Billy *et al*. (2001) indicated that auxin phytohormone is necessary through the development of nodule primordial and of the vasculature within the nodules of *Medicago truncatula*.

The number of nodules varied according to the stage of contribution of the GA3 phytohormone (Table 6). The highest level of nodulation was found with the GA3 contribution (0.1 g/l) at post-flowering. Ferguson *et al*. (2005) postulated that the application of an exogenous GA3 increased significantly the nodules number of the mutants of *Pisum sativum* plants and also noted that the nodule formation of these mutants is strictly controlled by the GA3 concentration. According to Rafique *et al*. (2021), the highest levels of nodules (16) and their dry biomass (0.22 g) were obtained by exogenous treatment of GA3 at 10^{-5} M combined with *Rhizobium* inoculation.

The analysis of results showed that only the 10^{-4} g/l IAB concentration at germination then at pre-flowering stages induced significantly leaves number in comparison with the control sample (Table 6). All GA3 phytohormone treatments resulted in a significant increase in the number of leaves compared with the untreated plant.

For the number of branches, a significant increase between treated and control plants were only observed in IBA treatment (10^{-4} g/l) at germination then at pre-flowering stages and at post-flowering stage (Table 6). All treatments of plants with GA3 phytohormone increased significantly ($P<0.05$) this parameter compared to the untreated plant. The highest number of branches was noted for the 0.1 g/l GA3 treatment on pre-flowering. At the cellular level, the auxin responses were dependent upon the presence of Auxin Response Factors (AFRs) transcription factors and Auxin/Indole-3-Acetic Acid (Aux/IAA). These proteins are determined on their capacity to bond to promoter elements that confer auxin responsive gene expression (Emenecker and Strader, 2020).

No significant variation was observed in fresh weight of the aerial part of the plant between treated and non-treated plants among the different IBA phytohormone treatments (Table 6). The fresh weight of the aerial part of the plant was significantly raised under treatment with GA3 phytohormone at post-flowering stage (0.1 g/l and 0.2 g/l) and at pre-flowering stage (0.2 g/l). Dry weight of the aerial part of the plant was significantly improved with IBA phytohormone treatment (10^{-4} g/l and 10^{-3} g/l) at germination then at pre-flowering stages and at pre-flowering stage (Table 6). However, dry weight was significantly higher in the all GA3 hormone-treated plants as compared to the controls ones. Overall, the vegetative growth parameters of chickpea such as number of branches, fresh and dry weight of the aerial part and leaves number, were increased in plants treated with IBA and GA3 in certain treatment stages. According to Mousavi *et al*. (2016), the β-carotene content and cell growth of *Dunaliella salina,* were positively affected by indole-3-acetic acid at 1 µM. Zayed *et al*. (2017) postulated that the growth parameters such as leaf area and shoot and root fresh and dry weight were increased in sunflower plant treated with indole-3-acetic acid (auxin).

No significant increase in flowers number between treated and control plants, was observed in different IBA phytohormone treatments (Table 6). The highest flowers number was found with 10^{-4} g/l IBA at germination then at pre-flowering stages. The seeds number was significantly raised at IBA phytohormone treatments compared with the control plants (Table 6). The treatment of chickpea plants with IBA phytohormone (10^{-4} g/l and 10^{-3} g/l concentrations at different stages) increased significantly the weight of

Table 4: Variation in radicle length (cm) according to hormonal treatment (IBA) from 3 to 12 days after germination in Petri dishes.

Concentrations of IBA (g/l)	3 days	6 days	9 days	12 days
0	3.64c±0.08	3.9b±0.06	5.2c±0.09	6.7c±0.09
10^{-5}	4.8d±0.03	6.16c±0.14	7.44d±0.02	8.1d±0.20
10^{-4}	4.76d±0.08	6.44c±0.07	7.68d±0.13	9.32e±0.14
10^{-3}	3.06b±0.17	3.82b±0.16	4.8b±0.09	5.28b±0.12
10^{-2}	1.28a±0.14	1.76a±0.11	2.52a±0.17	2.7a±0.11

Average values with the same letter per column are not significantly different (P=0.05, Duncan test).

Table 5: Variation in radicle length (cm) according to hormonal treatment (GA3) from 3 to 12 days after germination in Petri dishes.

Concentrations of GA3 (g/l)	3 days	6 days	9 days	12 days
0	3.64bc±0.06	3.9a±0.09	5.2b±0.09	6.7c±0.13
0.05	2.9a±0.13	4.1ab±0.09	4.56a±0.07	4.56a±0.04
0.1	3.48b±0.08	4.36bc±0.11	5.04b±0.04	5.66b±0.08
0.15	3.7bc±0.05	4.08b±0.04	4.94b±0.10	5.48b±0.09
0.2	3.82c±0.05	4.38c±0.11	5.62c±0.17	7.42d±0.04

Average values with the same letter per column are not significantly different (P= 0.05, Duncan test).

Table 6: Phenological and productive parameters exhibited differently responses of chickpea to phytohormone treatments (GA3 and IBA) in pots.

Hormone treatments	NN	H (cm)	NR	FW (g)	DW (g)	NF	NL	NS	WS (g)
Control	18.2ab±4.82	26.7a±0.98	3.8a±0.20	4.25a±0.66	1.45ab±0.17	2a±0.00	42.6a±4.82	3.2ab±0.37	1.74bc±0.15
IBA11	35ce±5.43	30ab±0.89	6be±0.32	4.43ab±0.63	2.07cd±0.25	5.2ac±1.39	69.6c±4.63	7.6d±1.44	2.89de±0.58
IBA12	36.4ce±4.34	32.76b±1.40	5ac±0.71	5.63ac±0.99	2.24cd±0.29	4.6ab±0.24	60.2ac±7.83	6.8d±0.97	4.08f±0.43
IBA13	37ce±4.96	28.3a±0.80	6be±0.55	4.17a±0.42	1.86ac±0.08	5ac±0.32	57.2ac±2.82	7.4d±0.68	2.7cd±0.46
IBA21	39.6de±3.57	30.67ab±0.93	4.8ab±0.66	4.83ac±0.72	2bd±0.18	3.8ab±0.86	57ac±7.77	7.8d±0.92	5.46g±0.66
IBA22	40.2de±3.38	32.1b±0.80	5.4ac±0.40	5.21ac±0.31	2.05cd±0.16	4ab±0.63	58.4ac±3.25	6.6d±0.24	3.75ef±0.29
IBA23	41.4e±3.14	28a±0.32	4.2a±0.49	4.07a±0.30	1.41a±0.16	3.4ab±0.51	50ab±1.97	6cd±0.32	3.48de±0.28
GA11	25.2ac±5.15	51.85e±0.93	7.4ef±0.24	4.9ac±0.15	2.57de±0.13	8bd±0.51	61.8bc±3.61	3.8ab±0.20	1.11ab±0.15
GA12	14.8a±1.32	45d±1.61	7.6f±0.81	4.54ab±0.52	2.35cd±0.29	16.4e±2.29	62.6bc±7.63	3.8ab±0.37	1.07ab±0.18
GA13	29be±3.18	46.5d±1.43	5.8bd±0.58	6.51c±0.70	2.54de±0.19	10.8d±1.24	66.4bc±7.57	3.6ab±0.40	2.53cd±0.22
GA21	24ac±4.82	53.8ef±1.24	7.2cf±0.37	5.4ac±0.18	2.96e±0.10	8.4bd±0.68	62.4bc±3.60	2.6a±0.24	1.04ab±0.12
GA22	15.8ab±1.59	56.4f±2.30	7.2cf±0.97	8.6d±0.49	3.61f±0.06	10.2cd±0.49	57.6ac±4.42	2.4a±0.24	0.45a±0.05
GA23	27ad±1.14	41.5c±1.73	6.4cf±0.51	6.22bc±0.28	2.55de±0.15	28f±6.63	62.6bc±5.51	4.8bc±0.49	2.65cd±0.51

NN= Nodules number; H= Height of plant; NR= Number of ramifications; FW= Fresh weight of aerial part; DW= Dry weight of aerial part; NF= Number of flowers; NL= Number of leaves; NS= Number of seeds; WS= Weight of seeds.

-IBA11: [IBA]=10^{-4} g/l at germination then at pre-flowering stages; IBA12: [IBA]=10^{-4} g/l at pre-flowering stage; IBA13: [IBA]=10^{-4} g/l at post-flowering stage.

-IBA 21: [IBA]=10^{-3} g/l at germination then at pre-flowering stages; IBA 22: [IBA]=10^{-3} g/l at pre-flowering stage; IBA 23: [IBA]=10^{-3} g/l at post-flowering stage.

-GA11: [GA3]=0.1 g/l at germination then at pre-flowering stages; GA12: [GA3]= 0.1 g/l at pre-flowering stage; GA13: [GA3]= 0.1 g/l at post-flowering stage.

-GA21: [GA3]=0.2 g/l at germination then at pre-flowering stages; GA22: [GA3]=0.2 g/l at pre-flowering stage; GA23: [GA3]=0.2 g/l at post-flowering stage.

Average values with the same letter per column are not significantly different (P=0.05, Duncan test).

seeds (Table 6). Compared to the control, the seeds weight of plants treated by 10^{-3} g/l IAB at germination then at pre-flowering stages was 3-fold higher. Hasami and Abdi (2010) reported that application with Naphthaleneacetic acid (synthetic auxin) at 100 ppm, caused appreciable and significant increases in physical properties (Fruit weight, height, diameter and size) of date palm.

We note that all treatments with GA3 raised significantly the flowers number compared to the untreated plant. In addition, the weight and the number of seeds were increased in GA3 hormone-treated plants at post-flowering. Rady *et al*. (2021) revealed that foliar application of gibberellic acid at 20 mg/l in faba bean increased the growth, green pod yield and water use efficiency. Perumal *et al*. (2021) suggested that the application of GA3 significantly improved the average fruit yield in plant of kinnow (*Citrus reticulate Blanco*). Moreover, Valleser (2023) working in pineapple plant found that the presence of auxin, gibberellin and cytokinin promoted fruit size and quality.

CONCLUSION

The plant hormones are extremely important agent in plant growth and development processes. In this regard, the current study was conducted with an aim to evaluate the effects of two phytohormones (indole butyric acid and gibberellins) on some growth parameters of chickpea. Generally, the tested phytohormones increased the phenological and productive parameters of chickpea (radicle length, nodules number, plant height, ramifications number, fresh and dry weight of the aerial part of the plant, flowers number, leaves number, seeds number and seeds weight). However, the choice of phytohormone concentration and the stage of its application are useful in order to exploit the beneficial effects on promoting growth in chickpea plants. Our study will also profit future research on examining the physiological mechanisms of phytohormones action in chickpea growth.

Authors' contribution

Taoufik Hosni carried out the experiments, performed the analysis of results and wrote the manuscript. Zouhaier Abbes, Siwar Thebti, Mohamed Kharrat discussed the results and contributed to the final version of the manuscript and Ali Dahmane supervised this study.

Conflict of interest

All authors state that it is an original article and the data presented have neither been published in part nor under consideration for publication in any other journal. All the authors declare no conflicts of interest regarding this article.

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