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ENHANCING *CYCAS REVOLUTA* THUNB. GROWTH BY CALCIUM CARBONATE NANOPARTICLES UNDER WATER DEFICIT STRESS CONDITION

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ABSTRACT: The present work was conducted to study the effect of different Lithovit (nano-$CaCO_3$) rates 0, 2, 4, 6, 8 and 10 g/l and irrigation intervals (1 week and 2 weeks) on enhancing growth *Cycas revoluta,* Thunb. seedlings, that (one year old) during two experiments of 2018 and 2019 in Antoniades Research Branch, Horticulture Research Institute, Alex., Egypt. The obtained results cleared that the two irrigation intervals had significant effects on cycas growth during the two seasons. Lithovit (nano-$CaCO_3$) with concentrations of (0, 2, 4, 6, 8 and 10 g/l) under the two irrigation intervals caused significant increment in leaf length (cm), number of leaves per plant, leaves dry weight (g), stem circumference, stem dry weight (g), and root dry weight. Also, Lithovit (nano-$CaCO_3$ enhanced the leaves contents of chlorophyll a, b and total chlorophyll, total carbohydrate, and N%. Meanwhile, there was a significant decrease in proline and electrolyte leakage (EL) % contents. The optimum concentration of nano-$CaCO_3$ to enhance *Cycas revoluta* growth under deficit water was 8 and 10 g/l in plants irrigated every week followed by 10 g/l in plants irrigated every two weeks. Finally, it can be concluded that foliar application of nano-$CaCO_3$ can reduce the adverse effects of drought on cycas plants.

Key words: *Cycas revoluta*, irrigation intervals, Lithovit, nanoparticles.

INTRODUCTION

Cycas revoluta, Thunb. the common name is Sago palm, which belongs to the family Cycadaceae (Cycad family) (Bailey, 1960). Cycads are mostly traded as ornamental plants, with almost 59 million plants traded between 2002 and 2014. Of all these exports, more than 90% belong to *Cycas revoluta*. For ornamental products, the four main traded products of cycas are: whole plants; leaves; roots; and stems (CITES, 2016).

For many decades, *Cycas revoluta* has been cultivated and is one of the few species now cultivated in a sufficient amount to attain its demand. (Donaldson, 2003). This is an amazing plant for an indoor, outdoor container plant, and it is grown as bonsai in Asia, especially in Japan. The natural extract of *Cycas revoluta* is useful medicinal value, effectively inhibited gastric cancer cell growth and enhanced the anti-cancer effect (Xing-Liang *et al*., 2019).

Nano-fertilizers have recently been used for a slow release and productive use by the plants as an alternative to traditional fertilizers. It may improve the efficiency of nutrient usage and reduce environmental protection costs (Naderi and Shahraki, 2013 and Byan, 2020). Lithovit (nano-$CaCO_3$) effect is that calcium carbonate ($CaCO_3$) breaks down to calcium oxide (CaO) and carbon dioxide (CO_2) in clears out stomata, and this CO_2 increase the rate of photosynthesis, leading to increased carbon take-up and assimilation of carbon, thus enhancing plant development (Nassar *et al*., 2018). Lithovit nano-fertilizer particles contains calcium-magnesium carbonate combined with various essential elements needed for plant growth (N, P, K, Fe, Zn, Mn, Cu, sulphate and selenium).

A water stress is the most important factor influencing crop yield, it is necessary to get the maximum agricultural yield by the use of available water to get the greatest benefits from the unit area because existing agricultural land and irrigation water is rapidly decreasing (Zandi and Azarnivand, 2012). It was found that irrigating cotton plants every 21 days and spraying with Lithovit at the rate of 7.5 g\l, irrigation intervals and the interaction between nano-particles had a major impact on N, K and total sugars levels in leaves in both seasons and on total carbohydrates concentration in the second season (Attia *et al*., 2017).

The rate of budding due to cycads is relatively low, so that its high planting costs urgently require a new approach to solve technical problems. April and Thomas (2016) indicated that *Cycas revoluta* is the subject of more published cycad research than any other cycad species.

The aim of the present work is to study the effects of different concentrations of nano-$CaCO_3$ on *Cycas revoluta* plants under conditions of two irrigation intervals to reach the best growth rates.

MATERIALS AND METHODS

The experimental trial was carried out through the two successive seasons of 2018/2019 and 2019/2020 in Antoniadis Research Gardens, Horticulture Research Institute, Alex., Egypt. One-year-old seedlings with 2 leaves (10 - 12 cm length) of *Cycas revoluta* were grown in a mixture of soil and sand (1:1 v/ v) in 25 cm diameter clay pots under natural light in the plastic greenhouse condition. Some chemical and physical properties analysis of the used mixture is illustrated in Table (1).

On the 5th March, 2018 and 3rd March, 2019 in both seasons respectively, 6 concentrations of nano-$CaCO_3$ (0, 2, 4, 6, 8 and 10 g/l) applied as a foliar spray repeated once every two weeks (till the end of the experiment), for foliar spray, all rates were applied using a hand sprayer and the wetting agent tween-20 was added to each test solution. Each plant was sprayed individually till the point of run-off, under two irrigation intervals (every 1 week and 2 weeks) as well as their interactions on *Cycas revoluta* plants. The different components of Lithovit were clarified in Table (2)

The complete fertilizer of 19-19-19 was top-dressed (2.5 g/pot) at three- week interval, throughout the period from March, 2018 till July, 2020.

Statistical analysis:

This experiment was factorial

Table 1. Chemical analysis of the used mixture soil for the two growing seasons.

pH	EC dS/m	Cations (meq/l)				Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{--}
8.11	1.69	1.85	0.75	1.48	0.38	1.13	2.11	1.03

Table 2. Chemical analysis of Lithovit.

Components	Value (%)	Components	Value (%)
Calcium carbonate	79.19	**Selenium dioxide**	11.41
Nitrogen	0.06	**Iron**	1.31
Phosphate	0.01	**Manganese**	0.014
Potassium oxide	0.21	**Zinc**	0.005
Magnesium carbonate	4.62	**Copper**	0.002
Sulphate	0.33	**Clay**	0.79

experiment (2 irrigation intervals × 6 Lithovit nano-$CaCO_3$ concentration), and conducted using a complete randomized block design which included three replicates, for each treatment, three pots were used as an experimental unit for each replication. According to Snedecor and Cochran (1989), the means of the individual variables and their interactions were contrasted by the L.S.D test at 5%.

Data recorded:

At the end of the experiment, on the 4th of July, 2019 and 9th of July, 2020 (in the first and second seasons; respectively) the experiments were terminated, and the following measurements and chemical analysis were determined.

1. Morphological measurements:

Leaf length (cm), number of leaves per plant, leaves dry weight (g), stem circumference, stem dry weight (g), and root dry weight (g).

2. Chemical analysis in leaves:

Total chlorophyll content (mg/100 g fresh weight) was determined according to Moran (1982), total carbohydrate content (% D.W.) according to Hodge and Hofreiter (1962), proline content (µg/g D.W) according to Bates *et al*. (1973) and determination of electrolyte leakage (EL%) by using the method described by Sullivan and Ross (1979). The chemical analysis terminated in new leaves.

RESULTS AND DISCUSSION

1. Morphological measurements:

Leaf length (cm):

The results of both seasons showed that the irrigation interval every week had a significant effect on increasing the leaf length as compared with the irrigation interval at 2 weeks, in both seasons and the shortest leaves were recorded at the irrigation every 2 weeks (26.29 and 26.70 cm) compared with (30.20 and 31.05 cm) for the irrigation every week, respectively (Table, 3).

The foliar application of Lithovit (nano-$CaCO_3$) at the different rates (2-10 g\l), in both seasons significantly increased the leaf length as compared with untreated plants. The increment in the leaf length due to Lithovit (nano-$CaCO_3$) was in proportional with the level of Lithovit (nano-$CaCO_3$), so plants treated with the dose at 10 g/l and 8 g/l had the largest leaves (33.84 - 35.33 cm) and (32.45 and 33.58 cm) in the 1st and 2nd seasons, respectively.

Regarding to the interaction between irrigation intervals and Lithovit (nano-$CaCO_3$) treatments, the results showed a significant effect on leaf length. The largest means were recorded with Lithovit (nano-$CaCO_3$) at the rates of 10 g/l (37.22 and 39.11cm) and 8 g/l (34.67 and 35.67cm) combined with weekly irrigation, in the 1st and 2nd seasons, respectively. The shortest leaf length was found in untreated plants irrigated every 2 weeks, in both seasons. Significant differences were detected among the rates of Lithovit (nano-$CaCO_3$), except among 6 g\l and (8 and 10) g\l nano- $CaCO_3$ for plants watered every 2 weeks, in the first season, also the same effect was observed in the second one between (6 g/l) nano-$CaCO_3$ watered every week and (8 and 10 g/l) nano-$CaCO_3$ watered every 2 weeks, (Table, 3).

Number of leaves per plant:

Table (3) presents the comparison between the two irrigation intervals which showed that, the weekly irrigation was superior to watering every two weeks, in increasing the number of leaves per plant, giving (4.98 and 4.96) leaves in the two seasons, respectively. With respect to the Lithovit (nano-$CaCO_3$) application, regardless the irrigation intervals; there was significant increment in the formation of leaves per plant compared to untreated plants. The rate of 10 g/l nano-$CaCO_3$ was able to increase the number of leaves to the maximum value as compared with the other Lithovit (nano-CaCO3) rates, in both seasons, and the differences between 8 and 10 g\l Lithovit (nano-$CaCO_3$) application

Table 3. Means of a leaf length (cm), number of leaves/plant and leaves dry weight (g) of *Cycas revoluta* as influenced by Lithovit (nano-$CaCO_3$) and irrigation intervals levels and their combinations between them, during the two seasons of 2018 and 2019.

Nano $CaCO_3$ (L)	Irrigation intervals (I) One Week	Two Weeks 2018	Means (L)	One Week	Two Weeks 2019	Means (L)
			Leaf length (cm)			
Nano $CaCO_3$ 0.0 g/l	23.81	19.76	21.78	24.16	20.75	22.46
Nano $CaCO_3$ 2.0 g/l	25.42	22.28	23.85	26.36	21.61	23.99
Nano $CaCO_3$ 4.0 g/l	28.20	25.38	26.79	29.78	26.61	28.20
Nano $CaCO_3$ 6.0 g/l	31.89	29.66	30.78	31.22	28.20	29.71
Nano $CaCO_3$ 8 .0 g/l	34.67	30.22	32.45	35.67	31.50	33.58
Nano $CaCO_3$ 10.0 g/l	37.22	30.45	33.84	39.11	31.55	35.33
Means (I)	30.20	26.29		31.05	26.70	
L.S.D. at 0.05 (I)		0.55			0.56	
L.S.D. at 0.05 (L)		0.94			0.97	
L.S.D. at 0.05 (I × L)		1.34			1.37	
			Number of leaves/plant			
Nano $CaCO_3$ 0.0 g/l	3.45	3.00	3.22	3.78	3.11	3.44
Nano $CaCO_3$ 2.0 g/l	3.89	3.67	3.78	4.00	3.56	3.78
Nano $CaCO_3$ 4.0 g/l	4.78	4.00	4.39	4.67	3.78	4.22
Nano $CaCO_3$ 6.0 g/l	5.55	4.78	5.17	5.44	4.67	5.06
Nano $CaCO_3$ 8 .0 g/l	6.00	5.00	5.50	5.78	4.55	5.17
Nano $CaCO_3$ 10.0 g/l	6.22	5.11	5.67	6.11	5.00	5.56
Means (I)	4.98	4.26		4.96	4.11	
L.S.D. at 0.05 (I)		0.21			0.23	
L.S.D. at 0.05 (L)		0.37			0.40	
L.S.D. at 0.05 (I × L)		0.52			0.57	
			Leaves dry weight (g)			
Nano $CaCO_3$ 0.0 g/l	13.19	10.38	11.79	15.70	9.95	12.82
Nano $CaCO_3$ 2.0 g/l	14.45	12.87	13.66	15.92	12.27	14.09
Nano $CaCO_3$ 4.0 g/l	16.06	14.10	15.08	16.01	14.40	15.20
Nano $CaCO_3$ 6.0 g/l	17.64	15.59	16.61	18.66	16.33	17.50
Nano $CaCO_3$ 8 .0 g/l	19.49	17.41	18.45	21.62	19.97	20.80
Nano $CaCO_3$ 10.0 g/l	21.62	18.88	20.25	22.11	19.46	20.78
Means (I)	17.07	14.47		18.34	15.40	
L.S.D. at 0.05 (I)		0.36			0.29	
L.S.D. at 0.05 (L)		0.63			0.49	
L.S.D. at 0.05 (I × L)		0.89			0.70	

were not significant in both seasons (Table, 3).

The interaction effect between irrigation intervals and the treatments of nano-$CaCO_3$ revealed that the maximum mean value was detected with the treatment of 10 g/l nano-$CaCO_3$ followed by 8 in cycas plants irrigated every week, with non-significant differences. Also, there was increment with the application of Lithovit (nano-$CaCO_3$) 8 and 10 g/l in plants watered every two weeks. It was noticed that the increment in the number of leaves in the treatments of 10, 8 and 6 g/l nano-$CaCO_3$ with irrigation interval per week was (5.67, 5.50 and 5.17) by (21.7%, 20.0% and 16.1%) respectively over the corresponding values of these treatments with irrigation every 2 weeks in the first season, and were (5.56, 5.17 and 5.06) by (22.2%, 27.0% and 16.5 %), respectively in the second one.

Leaves dry weight (g):

Dry weight of leaves was significantly affected by the different irrigation intervals, nano-fertilizer levels and their interaction in both trials as seen in Table (3). The results of the first season indicated that the highest average was detected with the treatment of 10 g/l nano-$CaCO_3$ followed by the treatments of 8 g/l nano- $CaCO_3$ in plants irrigated every week and 10 g/l nano- $CaCO_3$ in plants irrigated every two weeks. The data of second season revealed that the highest average was recorded in plants treated with 8 and 10 g/l Lithovit (nano-$CaCO_3$) combined with weekly irrigation, followed by 8 and 10 g/l nano-$CaCO_3$ combined with irrigation every 2 weeks. On the other hand, nano-$CaCO_3$ rates of 10 g/l nano-$CaCO_3$ in the first season and 10 and 8 g/l nano-$CaCO_3$ in the second one significantly recorded the heaviest dry weight of leaves compared with the other rates.

Stem circumference (cm):

The response of stem circumference of cycas seedlings to the application of Lithovit (nano-$CaCO_3$) under two regimes of irrigation are shown in Table (4). In both seasons the effects of irrigation interval, nano-$CaCO_3$ levels and interaction between them were significant. Considering the general effect of nano-$CaCO_3$ levels, it was noted, in both seasons, that the stem circumference was significantly increased with the increase of the nano-fertilizer rate. The highest average value, in both seasons, was recorded at nano-$CaCO_3$ rate of 10 g/l in plants irrigated every week, while the lowest one was noticed in irrigated every 2 weeks, while the lowest one was noticed in plants irrigated every 2 weeks with Lithovit (nano-$CaCO_3$) application.

Stem dry weight (g):

The comparison between the two irrigation intervals at any nano-$CaCO_3$ rate revealed that the stem dry weight of plants irrigated every week was significantly higher than that of plants irrigated every 2 weeks, Table (4). In the first season, the significant highest stem dry weight was recorded with the application of Lithovit (nano-$CaCO_3$) at the rate of 10 and 8 g/l combined with weekly irrigation, followed by the rate of 8 g/l nano- $CaCO_3$ combined with irrigation every two weeks, and in the second one, was recorded at the rate of 10 g/l nano- $CaCO_3$ irrigated every week. Regardless of irrigation intervals, all treatments of Lithovit (nano-$CaCO_3$) (2-10 g/l) significantly increased the stem dry weight, compared with untreated plants, in both seasons.

Root dry weight (g):

The data presented in (Table, 4) show the mean values of root dry weight. Regarding to irrigation intervals, it was noticed that, the irrigating plants every week at any rate of Lithovit (nano-$CaCO_3$) had markedly heavier root dry weight heaviest than that of plants irrigated every 2 weeks, through the two seasons. The heaviest root dry weights were achieved at the irrigation every week combined with the treatment of 10 g/l Lithovit (nano-$CaCO_3$) in the first and second seasons.

Table 4. Means of stem circumference (cm), stem dry weight (g) and root dry weight (g) of *Cycas revoluta* as influenced by Lithovit (nano-$CaCO_3$) and irrigation intervals levels and their combinations between them, during the two seasons of 2018 and 2019.

Nano $CaCO_3$ (L)	One Week	Two Weeks 2018	Irrigation intervals (I) Means (L)	One Week	Two Weeks 2019	Means (L)
			Stem circumference (cm)			
Nano $CaCO_3$ 0.0 g/l	11.48	7.00	9.24	11.19	6.95	9.07
Nano $CaCO_3$ 2.0 g/l	12.47	8.90	10.69	11.90	8.97	10.43
Nano $CaCO_3$ 4.0 g/l	13.38	10.57	11.97	12.47	10.71	11.59
Nano $CaCO_3$ 6.0 g/l	15.05	12.59	13.82	14.88	11.38	13.13
Nano $CaCO_3$ 8 .0 g/l	16.61	13.84	15.23	15.65	13.98	14.82
Nano $CaCO_3$ 10.0 g/l	17.22	14.39	15.80	16.21	14.21	15.21
Means (I)	14.37	11.21		13.71	11.03	
L.S.D. at 0.05 (I)		0.19			0.24	
L.S.D. at 0.05 (L)		0.32			0.42	
L.S.D. at 0.05 (I × L)		0.46			0.59	
			Stem dry weight (g)			
Nano $CaCO_3$ 0.0 g/l	13.70	11.53	12.62	12.80	10.99	11.90
Nano $CaCO_3$ 2.0 g/l	15.17	13.15	14.15	15.11	11.09	13.10
Nano $CaCO_3$ 4.0 g/l	17.36	14.60	15.98	14.97	11.02	13.50
Nano $CaCO_3$ 6.0 g/l	17.35	16.17	19.79	17.15	13.36	15.26
Nano $CaCO_3$ 8 .0 g/l	20.93	17.82	19.73	18.73	17.44	18.08
Nano $CaCO_3$ 10.0 g/l	19.72	17.41	18.57	20.13	16.57	18.35
Means (I)	17.37	15.11		16.48	13.58	
L.S.D. at 0.05 (I)		0.64			0.46	
L.S.D. at 0.05 (L)		1.12			0.80	
L.S.D. at 0.05 (I × L)		1.58			1.13	
			Root dry weight (g)			
Nano $CaCO_3$ 0.0 g/l	8.69	6.24	7.47	8.18	7.09	7.63
Nano $CaCO_3$ 2.0 g/l	8.90	7.09	8.00	8.90	8.29	8.60
Nano $CaCO_3$ 4.0 g/l	10.12	9.04	9.58	10.59	9.50	10.05
Nano $CaCO_3$ 6.0 g/l	12.37	11.02	11.70	12.37	10.97	11.67
Nano $CaCO_3$ 8 .0 g/l	15.20	12.86	14.03	15.47	12.46	14.05
Nano $CaCO_3$ 10.0 g/l	16.92	13.12	15.02	17.52	13.20	15.36
Means (I)	12.03	9.90		12.17	10.28	
L.S.D. at 0.05 (I)		0.35			0.33	
L.S.D. at 0.05 (L)		0.61			0.58	
L.S.D. at 0.05 (I × L)		0.86			0.81	

Using all nano-fertilizers rates increased dry weight of root as compared with the untreated plants in both seasons. In this respect, applying the highest rate of nano-$CaCO_3$ at 10 g/l revealed its superiority for producing the heaviest root in both seasons. The results of increasing root dry weight are similar to those mentioned by Farooq *et al*. (2010); Liu *et al.* (2011) and Sajyan *et al*. (2018). From the results described above and by testing the vegetative and root characters of *cycas revoluta*, it is treated with nano-$CaCO_3$ treatments as a foliar spray under two irrigation intervals, there was an increment in leaf length, number of leaves per plant, leaves dry weight, stem circumference, stem dry weight and root dry weight. Similar results were mentioned by Nassef and Nabeel (2012); Abo El-Hamd and Abd Elwahed (2018); Ghatas and Mohamed (2018); Morsy *et al.* (2018) and Byan (2020). The results show superior growth of plants that were irrigated every week to plants that were irrigated every two weeks. In this regard, Yurany *et al*. (2016) pointed out that the oil palm plants respiration under water deficit was 87%, higher than that registered under field capacity. The field capacity enhanced vegetative growth, and the water deficit significantly affected the plant height, leaves number and total dry weight. Moreover, Jazayeri *et al.* (2015) indicated that water deficit damage all aspects of the metabolism of the oil palm, then restricted the growth regulators and photo assimilates production. On the other hand, the stomata closure naturally affects more processes than just transpiration: the restriction of CO_2 uptake by leaves is closely related to the stomatal water loss control. The reducing of net carbon assimilation/photosynthetic rate (PN) and the reduction in intercellular CO_2 concentration are thus considered as another early symptom of water deficit. In the initial stages of drought, the minimizing effect of stomatal closure on rate of transpiration is larger than the impact of CO_2 assimilation, but with more development of water stress, both processes are often dramatically decreased (Benešová, 2012). Reductions in leaf area (number and size) and stomatal closure, impaired carboxylation enzyme, ATP synthesis activities, and photosynthetic apparatus destruction among the key factors lowering carbon fixation under drought (Barlow, 1988; Yamance, *et al*. 2003). Miyashita *et al.* (2005) indicated that reduce photosynthesis and transpiration rates were due to reduce stomatal conductance in kidney beans (*Phaseolus vulgaris* L). Likewise, drought stress gradually decreased CO_2 assimilation rates because of reduced stomatal conductance in (*Cocos nucifera* L.) coconut plants (Gomes *et al.*, 2010). Lithovit (a nano-$CaCO_3$) acts as a long-term reservoir supplying CO_2 to the plants; it can therefore increase plant growth by increasing photosynthesis net since higher CO_2 can suppress oxygenase activity of ribulose-1, 5-bisphosphate; decrease photorespiration; and increase carbon assimilation for plant growth and production. Activity of oxygenase reduced photorespiration; and increased assimilation of carbon for growth and production of plants. Via increased carbon assimilation, biomass and leaf area of plants, elevated CO_2 concentrations generally increase plant growth (Abd El-Aal and Eid, 2018). Morsy *et al.* (2018) indicated that the irrigation levels and Lithovit (nano-$CaCO_3$) concentrations had significant effects on most yield traits, fertilizer use efficiency and quality of wheat plants. Increasing irrigation levels from 60% to 100% increased all agronomic traits. The nano-fertilizer treatment produced high values of all wheat yield parameters. Also, in this regard, El-Shazly and Abd El-All (2019) reported that foliar CO_2 fertilizer as a 7.5 g/l nano-$CaCO_3$ significantly reduced leaf water deficient osmotic pressure and plasma membrane permeability and significantly increased leaves total water and relative water contents. In this respect, Byan (2020) indicated that the common bean plants which sprayed with Lithovit (nano-$CaCO_3$) and combined with 100% or 75% of irrigation field capacity gave stimulatory effects on all vegetative growth characteristics compared with the untreated treatment. With respect to Lithovit (nano-$CaCO_3$), a natural enhanced foliar CO_2 fertilizer will significantly increase the rate of photosynthesis and influence the physiological process of plants and increase growth. The field capacity favored vegetative growth, and the water deficit significantly affected the number of leaves, plant height, total dry weight, while the water deficit conditions generated the most pressure to reduce these variables.

2. Chemical composition of leaves:

Chlorophyll contents (mg/100 g fresh weight):

The examined treatments and the interaction between them gave a significant effect on cycas leaves chlorophyll contents, the weekly irrigation significantly increased chlorophyll amounts, compared with the irrigation every 2 weeks.

Table (5) illustrated that the leaf chlorophyll a, b ant total chlorophyll contents were significantly increased in the treated plants, compared with the non-treated ones, The highest amount of chlorophyll a, b and a + b was noticed at the irrigation interval every week combined with Lithovit (nano-$CaCO_3$) at the rate of 10 g/l, followed by the rate of 8 g/l nano- $CaCO_3$ in plants irrigated every week in the first season and then 10 g/l in plants irrigated every 2 weeks in the second one, in the case of chlorophyll a + b.

The general effect of Lithovit (nano-$CaCO_3$) rates exhibited significant increments of chlorophyll a, b, and a + b contents from 2 to 10 g/l nano-$CaCO_3$, compared with untreated plants (Table, 6).

Total carbohydrate content (%):

Applying Lithovit (nano-$CaCO_3$) as a foliar spray to plants grown under 1 week and 2 weeks irrigation intervals significantly increased the leaves content of total carbohydrates with increasing the rates. The highest mean of total carbohydrates was recorded with the Lithovit (nano-$CaCO_3$) at the rate of 10 g/l followed by 8 g/l, in the first season and recorded with Lithovit (nano-$CaCO_3$) at rate of 10 g/l and 8 g/l combined with weekly irrigation followed by 6 g/l nano-$CaCO_3$ irrigated every week and 10 g/l irrigated every 2 weeks (Table, 6). The lowest amount of the total carbohydrates was detected in the untreated plants combined with 2 weeks irrigation.

The effect of Lithovit (nano-$CaCO_3$), regardless of the irrigation intervals, shows that the amount of total leaves carbohydrate was significantly increased with increasing the Lithovit (nano-$CaCO_3$) rates except in the second season, there was non-significant difference between 10 and 8 g/l (Table, 6).

Proline concentration (µg/g):

Regarding to the effect of treatments of Lithovit (nano-$CaCO_3$) and irrigation regime on proline accumulation of cycas plants, the results in Table (6) indicated that the treatment of Lithovit (nano-$CaCO_3$) at 10 g/l followed by 8 g/l nao-$CaCO_3$ combined with weekly irrigation regime showed the lowest content of proline in the first season, and in the second one, it was observed with of 8 and 10 g/l Lithovit (nano-$CaCO_3$) followed by 6 g/l combined with weekly irrigation, where the greatest mean was obtained from treating the plants with the combination between 0 g/l nano-$CaCO_3$ and irrigation every 2 weeks followed by 1 g/l then 2 g/l nao-$CaCO_3$; respectively in the two seasons.

Regardless the irrigation interval, the highest mean of proline content resulted from untreated plants, however, the lowest values were resulted from the foliar application of Lithovit (nano-$CaCO_3$) at 10 g/l nao- $CaCO_3$ in both seasons. Irrigation intervals exhibited significant differences in leaves concentrations of proline in both seasons. The minimum values of proline obtained in plants which irrigated every 1 week as compared with the plants which irrigated every 2 weeks.

Electrolyte leakage (EL) %:

Table (7) demonstrated that in leaves of cycas plants treated with all levels of Lithovit (nano-$CaCO_3$), there was a decrease

Table 5. Effect of Lithovit (nano-$CaCO_3$) concentrations and irrigation intervals on chlorophyll a, b and chlorophyll a + b (mg/100 g fresh weight), of *Cycas revoluta* leaves during 2018 and 2019 growing seasons.

Nano $CaCO_3$ (L)	Irrigation intervals (I) One Week	Two Weeks 2018	Means (L)	One Week	Two Weeks 2019	Means (L)
			Chl. a			
Nano $CaCO_3$ 0.0 g/l	29.70	22.19	25.95	24.95	20.47	22.71
Nano $CaCO_3$ 2.0 g/l	34.99	27.04	31.01	32.71	24.86	28.79
Nano $CaCO_3$ 4.0 g/l	39.34	35.19	37.26	36.43	31.38	33.91
Nano $CaCO_3$ 6.0 g/l	49.42	41.69	45.56	44.89	34.18	39.54
Nano $CaCO_3$ 8 .0 g/l	60.28	56.52	58.40	56.04	48.79	52.42
Nano $CaCO_3$ 10.0 g/l	65.52	59.81	62.67	59.17	51.47	55.32
Means (I)	46.54	40.41		42.36	35.19	
L.S.D. at 0.05 (I)		0.50			0.87	
L.S.D. at 0.05 (L)		0.87			1.50	
L.S.D. at 0.05 (I × L)		1.23			2.12	
			Chl. b			
Nano $CaCO_3$ 0.0 g/l	12.08	14.07	13.39	12.77	14.10	13.05
Nano $CaCO_3$ 2.0 g/l	17.64	13.41	15.53	16.08	13.80	14.94
Nano $CaCO_3$ 4.0 g/l	21.75	13.90	17.83	19.95	15.23	17.59
Nano $CaCO_3$ 6.0 g/l	24.30	15.16	19.73	22.72	17.82	20.27
Nano $CaCO_3$ 8 .0 g/l	27.82	20.16	23.99	26.10	19.17	22.63
Nano $CaCO_3$ 10.0 g/l	27.91	20.96	24.43	27.45	20.50	23.98
Means (I)	21.92	16.28		20.84	16.77	
L.S.D. at 0.05 (I)		0.25			0.17	
L.S.D. at 0.05 (L)		0.44			0.29	
L.S.D. at 0.05 (I × L)		0.62			0.41	
			Chl. a + b			
Nano $CaCO_3$ 0.0 g/l	44.92	34.82	39.87	37.60	34.03	35.82
Nano $CaCO_3$ 2.0 g/l	52.44	39.24	45.84	46.63	36.99	41.81
Nano $CaCO_3$ 4.0 g/l	61.34	48.15	54.75	53.81	44.83	49.32
Nano $CaCO_3$ 6.0 g/l	73.90	55.80	64.85	62.57	53.72	58.15
Nano $CaCO_3$ 8 .0 g/l	85.54	77.69	81.61	62.33	61.65	61.99
Nano $CaCO_3$ 10.0 g/l	93.78	78.67	86.23	77.71	70.60	74.15
Means (I)	68.65	55.73		56.78	50.30	
L.S.D. at 0.05 (I)		0.32			0.28	
L.S.D. at 0.05 (L)		0.56			0.49	
L.S.D. at 0.05 (I × L)		0.79			0.69	

Table 6. Effect of Lithovit concentrations and irrigation intervals on total carbohydrates % and proline concentration (µg/g) of *cycas revoluta* leaves during 2018 and 2019 growing seasons.

Nano $CaCO_3$ (L)	Irrigation intervals (I) One Week	Two Weeks 2018	Means (L)	One Week	Two Weeks 2019	Means (L)
			Total carbohydrate %			
Nano $CaCO_3$ 0.0 g/l	12.14	9.86	11.00	13.88	10.49	12.19
Nano $CaCO_3$ 2.0 g/l	14.90	11.94	13.42	15.09	13.66	14.38
Nano $CaCO_3$ 4.0 g/l	17.83	15.77	16.80	16.13	16.16	16.15
Nano $CaCO_3$ 6.0 g/l	19.22	16.53	17.87	20.67	17.22	18.94
Nano $CaCO_3$ 8 .0 g/l	20.19	17.68	18.94	22.41	17.85	20.13
Nano $CaCO_3$ 10.0 g/l	21.06	18.57	19.81	22.98	18.01	20.49
Means (I)	17.56	15.06		18.53	15.57	
L.S.D. at 0.05 (I)		0.31			0.24	
L.S.D. at 0.05 (L)		0.54			0.42	
L.S.D. at 0.05 (I × L)		0.76			0.60	
			Proline conc. (µg/g)			
Nano $CaCO_3$ 0.0 g/l	20.24	28.17	24.20	19.21	25.81	22.51
Nano $CaCO_3$ 2.0 g/l	18.12	26.19	22.16	17.43	22.74	20.09
Nano $CaCO_3$ 4.0 g/l	17.08	25.37	21.22	15.29	20.05	17.67
Nano $CaCO_3$ 6.0 g/l	14.70	22.50	18.60	13.47	17.15	15.31
Nano $CaCO_3$ 8 .0 g/l	12.88	17.01	14.95	12.42	15.76	14.09
Nano $CaCO_3$ 10.0 g/l	11.12	15.50	13.31	12.02	14.39	13.20
Means (I)	15.69	22.46		14.97	19.32	
L.S.D. at 0.05 (I)		0.28			0.17	
L.S.D. at 0.05 (L)		0.48			0.30	
L.S.D. at 0.05 (I × L)		0.67			0.43	

in electrolyte leakage. The plants irrigated every 2 weeks show significant increase in electrolyte leakage compared to plants which irrigated every week, in both seasons. Regardless irrigation intervals the increase of nano-$CaCO_3$ levels resulted in reduction of EL %, as the level of 10g/l nano-$CaCO_3$ recorded 36.5% and 38% reduction in EL % comparing with untreated plants, followed by 8 g/l nano-$CaCO_3$ which record 31.9% and 33.3%. The treatment of 10 g/l for plants irrigated every week (in both seasons) recorded the lowest mean of EL %, followed by 8 g/l, Lithovit (nano-$CaCO_3$) as well as the treatment of 10 g/l nano-$CaCO_3$ combined with irrigation every 2 weeks.

Nitrogen content N (%):

Table (7) shows the effect of foliar application of Lithovit (nano-$CaCO_3$), irrigation intervals and their interaction on nitrogen content. The results indicated that the N% content of the cycas leaves was increased with increasing the concentration of Lithovit (nano-$CaCO_3$) and the highest content was obtained at 10 g/l nano-$CaCO_3$ followed by 8 g/l which recorded 30% and 27% increase over control, in the first season and in the second one recorded 26% and 23%, respectively. Regard to the irrigation intervals, the irrigation every week recorded the highest value of N% content, in both seasons.

Table 7. Effect of Lithovit concentrations and irrigation intervals on electrolyte leakage (EL) % and N% of *Cycas revoluta* leaves during 2018 and 2019 growing seasons.

Nano $CaCO_3$ (L)	Irrigation intervals (I)					
	One Week	Two Weeks 2018	Means (L)	One Week	Two Weeks 2019	Means (L)
			EL (%)			
Nano $CaCO_3$ 0.0 g/l	36.25	43.37	39.81	37.42	45.61	41.52
Nano $CaCO_3$ 2.0 g/l	35.40	41.71	38.55	35.57	43.30	39.44
Nano $CaCO_3$ 4.0 g/l	32.56	38.49	35.53	31.78	37.74	34.76
Nano $CaCO_3$ 6.0 g/l	28.99	34.45	31.72	27.47	32.81	30.14
Nano $CaCO_3$ 8 .0 g/l	24.00	30.85	27.43	25.37	29.99	27.68
Nano $CaCO_3$ 10.0 g/l	21.87	28.68	25.27	23.02	28.48	25.75
Means (I)	29.84	36.26		30.11	36.32	
L.S.D. at 0.05 (I)		0.61			0.50	
L.S.D. at 0.05 (L)		1.05			0.87	
L.S.D. at 0.05 (I × L)		1.49			1.23	
			N %			
Nano $CaCO_3$ 0.0 g/l	1.58	1.38	1.48	1.63	1.47	1.55
Nano $CaCO_3$ 2.0 g/l	1.72	1.31	1.52	1.75	1.53	1.64
Nano $CaCO_3$ 4.0 g/l	1.79	1.57	1.68	1.85	1.61	1.73
Nano $CaCO_3$ 6.0 g/l	1.86	1.73	1.79	1.95	1.77	1.86
Nano $CaCO_3$ 8 .0 g/l	1.95	1.80	1.88	1.99	1.83	1.91
Nano $CaCO_3$ 10.0 g/l	2.02	1.83	1.93	2.04	1.85	1.95
Means (I)	1.82	1.60		1.87	1.68	
L.S.D. at 0.05 (I)		0.07			0.02	
L.S.D. at 0.05 (L)		0.12			0.04	
L.S.D. at 0.05 (I × L)		0.17			0.06	

Drought reduces significantly each root nutrient uptake and transport from the roots to the shoots, because of restricted transpiration rates and reduced transport and permeability of the membrane (Byan, 2020). Attia *et al.* (2017) reported that the application of nano-$CaCO_3$ at the rate of 7.5 g/l combined with irrigation cotton plants each 21 days resulted in significant positive reaction on chemical composition of cotton leaf. The results of Lithovit (nano-$CaCO_3$) treatments effect on chlorophyll contents were supported with those reported by Shallan *et al.* (2016), Attia *et al*. (2017), Doklega (2017), Ghatas and Mohamed (2018) and Sajian *et al*. (2019). In the same line, El-Shazly and Abd El All (2019) found that foliar CO_2 fertilizer as a foliar application of Lithovit (nano-$CaCO_3$) at a rate of 7.5 g/l significantly increased concentrations of photosynthetic pigments, such as chlorophyll a, chlorophyll b, total chlorophyll a + b and carotenoids. Attia *et al.* (2017) indicated that, leaves chlorophyl, b and carotenoids and leaves N, P, K contents were significantly increased in favor of applying CO_2 fertilizer (in the form of Lithovit). In this respect, Maswada and Abd El-Rahman (2014) examined the effect of foliar spray application with Lithovit (nano-$CaCO_3$-fertilizer) on growth, physiological of two wheat genotypes. Maximum total soluble sugars content was recorded by Lithovit (nano-$CaCO_3$). Reduction in proline accumulation in leaves as a result of nano-$CaCO_3$ application are in harmony with those obtained by Yadav *et al*. (2005), Attia *et al*. (2017), and El Shazly and Abd El All (2019). Accumulation of proline has been recommended for use as a parameter of selection for stress tolerance (Yancy *et al.*, 1982). Proline accumulation might respond to stresses such as temperature, drought and starvation (Sairam *et al.*, 2002). Under water stress, cell membranes are subjected to changes such as enlarge of permeability and reduce of selectivity, which can be seen through the enlarge in electrolyte leakage.

Results clearly showed that application of nano-$CaCO_3$ reduced the damage of drought stress which indicated by decreased membrane electrolyte leakage (EL). Similar results were obtained by Gonzalez-Mendozaa *et al*. (2009), Masoumi *et al.* (2010), El-Shazly and Abd El-All (2019), and Sajyan *et al*. (2018). Also, Masoumi *et al.* (2010) observed a general extend of electrolyte leakage under drought stress, which suggest the occurrence of damage to cell membranes. Under drought stress conditions compared with untreated plants of cotton plant, CO_2 fixation is decreased and photosynthetic pigments, carbohydrates accumulation and nitrogenous compounds were reduced Shallan *et al*. (2019).

Shallan *et al.* (2019) reported that treatments of Lithovit (nano-$CaCO_3$) significantly promote photosynthesis and explain that the natural content of CO_2 in the air limit photosynthesis. Lithovit (nano-$CaCO_3$) improved CO_2 gas inside the leaves of cotton plant, thus increased the photosynthesis and growth parameters, Emara *et al.* (2018).

Lithovit's function as a source of calcium carbonate reduced the formation of carbon dioxide accumulating in cells inside plant cells and increased the rate of photosynthetic assimilation and thus increased total sugars and total carbohydrates. Plants react to dry season push by closing stomata to restrain water misfortune, decreasing carbon stream, and diminishing ATP synthase. Treatment with proline and antioxidant agents caused stimulation of the photosynthetic pigments in chickpea plants. Down-regulated chlorophyllase action and up-regulated expression of chlorophyll biosynthetic qualities may result in expanded. There was a positive relationship between the stomatal conductance and transpiration in response to the lack of water availability. This conduct has been evaluated and suggested formerly under water deficit conditions (Silva *et al.*, 2013). These information are concordant with formerly suggested information for maize (Benešová *et al.,* 2012), soybean (Fenta *et al.,* 2012) and sugarcane (Silva *et al.*, 2013), wherein greater tolerant flowers confirmed much less inhibition of the transpiration and stomatal conductance and a smaller reduction of photosynthetic throughout drought condition (Jazayeri1 *et al.*, 2015).

The components of Lithovit (nano-$CaCO_3$) particles used as a foliar spray are highly small, which make it easy to enter through the leave stomata. These components play an important role of photosynthesis, improving plant metabolism witch increase growth and dry weight accumulation. Also, nano-$CaCO_3$ improved water use under drought stress, thus resulted in stimulation of photosynthesis rates, carbohydrates, N, P and K concentration (El-Shazly and Abd El-All, 2019). The application of a lot of CO_2 will increase plant water use potency and lead to less water use (Prior *et al.,* 2011). Elevated CO_2 stimulates photosynthesis resulting in enhanced carbon (C) uptake and assimilation, thereby, as increasing total carbohydrates and total sugars concentrations in leaves.

Calcium carbonate ($CaCO_3$) decomposes to carbon dioxide (CO_2) and calcium oxide (CaO) in stomata of leaves, and this carbon dioxide induces the intensity of photosynthesis. The role of Lithovit (nano-$CaCO_3$) as a source of calcium and carbonate which reduced inside plant cell to form carbon dioxide which accumulate in cells and increased the rate of photosynthetic assimilation and consequently increased total sugars and total carbohydrates. Calcium acts as a base for neutralizing organic acids generated during the growing process and aids in improving the translocation of carbohydrates from leaves to fruits and nitrogen absorption. Lithovit (nano-$CaCO_3$) contains magnesium that is the central element in chlorophyll molecule and plays an important role in many plant physiological processes such as photosynthesis, sugar synthesis, and starch translocation. N is an essential a part of chlorophyll production through photosynthesis (Tucker, 1999). Nitrogen performs an important function in CO_2 transformation to sugar (Uchida, 2000). Potassium plays a main role in plants exposed to drought stress, with respect to water relations and photosynthesis and K are important elements folded for physiological mechanisms of growth in the plant. Zinc in Lithovit (nano-$CaCO_3$) is required in the synthesis of tryptophan, a precursor of IAA synthesis, it is important for several biochemical procedures consisting of cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation and membrane integrity (Uchida, 2000). Phosphorus in Lithovit (nano-$CaCO_3$) (decomposes carbohydrates induction in photosynthesis; and it is involved in many other metabolic processes required for normal growth, such as photosynthesis, respiration and fatty acid synthesis and it promote early growth, where normal plant growth cannot be carried out without P. Also, sulphur in Lithovit (nano-$CaCO_3$) compound is important element in the synthesis of amino acids needed to create proteins. Sulfur and iron are important in chlorophyll induction and utilization of sulfur and alternative important nutrients. Cu in Lithovit (nano-$CaCO_3$) compound is part of the chloroplast protein plastocyanin.

CONCLUSION

The present study on *Cycas revoluta* demonstrated that the foliar application with CO_2 fertilizer in the form of Lithovit (nano-$CaCO_3$) under deficit water decreased the adverse effects of deficit water and enhanced growth compared with untreated plants. The plants under the two irrigation intervals which treated with 8 and 10 g/l nano-CaCO3 resulted the highest values of vegetative growth and the contents of leaves chlorophyll, total carbohydrate and N%, and reduced the contents of proline and value electrolyte leakage of leaves. The highest values of studied traits were obtained at 10 g/l and 8 g/l Lithovit (nano-CaCO3) combined with weekly irrigation as well as 10 g/l combined with irrigation every 2 weeks.

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تحسين نمو *Cycas revoluta,* Thunb. باستخدام نانو كربونات الكالسيوم تحت ظروف الإجهاد المائي

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قسم بحوث نباتات الزينة وتنسيق الحدائق، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر

تم تنفيذ هذا البحث بفرع بحوث نباتات الزينة بأنطونيادس، الإسكندرية، خلال الموسمين ٢٠١٨ و ٢٠١٩ لدراسة تأثير فترتين من الري (كل أسبوع وكل أسبوعين) والرش الورقي لسماد الليثوفيت (نانو كربونات الكالسيوم) بتركيزات صفر، ٢، ٤، ٦، ٨ و ١٠ جم/لتر، على تحسين النمو لنبات السيكس *Cycas revoluta* Thunb. (شتلات عمر عام). وقد أظهرت النتائج أن الرش الورقي لسماد ثاني أكسيد الكربون من خلال ليثوفيت (نانو- كربونات الكالسيوم) لنبات السيكس تحت ظروف الإجهاد المائي أدى الى تحسين النمو مقارنة بالنباتات غير المعاملة تحت نفس الظروف، النباتات المعاملة بالتركيز ٨ و ١٠ جم/لتر من سماد نانو كربونات الكالسيوم تحت ظروف كلا فترتي الري أدى الى الحصول على أعلى القيم لكلاً من النمو الخضري، محتوى الاوراق من الكلوروفيل أ ، ب و كذلك الكلوروفيل الكلي، و الكربوهيدرات الكلية، و النسبة المئوية للنيتروجين، بينما أدى تطبيق تلك المعاملات الى خفض النسبة المئوية للبرولين والتسرب الأيوني في الأوراق. سجلت أعلى قيم للصفات المدروسة عند ٨ و ١٠ جم/لتر مقترناً بالري مرة كل أسبوع الى جانب ١٠ جم/لتر مقترنا بالري كل اسبوعين.