

ORIGINAL ARTICLE

Raising growth of date palm cv. Bartomouda plantlets with date palm pollen grain extract and amino acids compound under greenhouse condition

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ABSTRACT

Plantlets of date palm under greenhouse is needed for enhancing by different fertilizers treatments, this process can be done by natural products have useful contents to plants and environmental Thus in order to increase the growth and development of date palm (*Phoenix dactylifera* L.) plantlets *in vitro* production, plantlets were exposed to date palm pollen grain extract and amino acids compound in the green house, studying various levels as (T0) 2.0 g/l NPK one time/2 weeks, (T1) 600 ppm extract date palm pollen grain + T0, (T2) 800 ppm extract date palm pollen grain + T0, (T3) 3 ml/l total amino acids + T0 and (T4) 6 ml/l total amino acids + T0, plantlets were subjected one time/2 weeks. The experiment was conducted with three replications in randomized complete design with four treatments in addition to control treatment. Results showed that plant height, leaves numbers/plantlet and fresh and dry weights of leaves were significantly affected and were increasing under two levels of extract date palm pollen grain more than compounds of amino acids at two levels compared to control treatment. Furthermore, leaves chlorophyll a and b, total indoles mg/g f.w., carbohydrates and amino acids mg/g f.w. were rising under two levels of date palm pollen grain extract and compound of amino acids with significant difference. High concentration of extract pollen grain date palm has the highest estimated results. Thus the useful effect of natural extract as date palm pollen grain extract is for enhancing growth, in addition to protection of environment and soils under decreased chemical fertilisers.

KEYWORDS date palm, extract, growth, greenhouse, indoles

INTRODUCTION

Date palm plantlets under greenhouse condition need to be enhanced by different treatment for speedy cultured in the open field and fruits production. In spite of plants were being supplied by sufficient chemical fertilizers these treatment led to harmful effect on the plants and soils. Therefore nowadays applying safe substances additionally has been recommended. Thus different natural extract is largely utilised in the agricultural practice. A lot of natural extracts and substances are affected on plant growth and its physiological activities (Leonard 2008). Pollen grains are widely used in Egypt for many purposes. Pollens are male reproductive cells which is rich in very important constituents i.e. phytochemicals and nutrients and carotenoids and flavonoids are also good source of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals (Basim et al. 2006; Kroyer and Hegedus 2001). Palm pollen grains from different Egyptian cultivars are rich in different amino acids from 0.147 to 83.64 mg g⁻¹, B1 ranged from 11 to 60 mg g⁻¹, B2 ranged from 15 to 260 mg g⁻¹ and B12 from 14 to 2316 mg g⁻¹. Palm pollen grains contents of moisture 28.8%, ash 4.57%, fibers 1.37%, fat 20.74%, protein 31.11% and carbohydrates 13.41%, are considered as a good

source of micro elements B, Se, Mo Co, Cu and Ni. Palm pollen grains have glycosides, lake volatile substances, several steroids, brassinosteroid (Hassan 2011, Bishr and Samar 2012, Almeida-Muradian et al. 2005). Applying algae extractions to the soil improves soil characteristics that have a positive impact on nutritional status of plants (Al-Gosaibi 1994). Growth performance of olive transplants was markedly enhanced as their root zoon partially replaced nitrogen by algal bulk (Abdel-Maguid et al. 2004), fresh weight of date palm cv. Khalas by spraying application of 5-aminolevulinic acid at 50–100 ppm (Al-Khateeb et al. 2006), positive effect on initial root development and plant growth with application of phytohormones extracted from organic matter (Naeem et al. 2006, Dauda et al. 2008), positive effect of algae extracts as a new bio-fertilizer containing macronutrients as well as micronutrients. Some growth regulators aspolyamines significantly affected shoot characteristics and leaves content of nutrient (Kowalski et al. 1999, Shaaban 2001, Abd El-moniem and Abdallah, 2008). Foliar application of biofertilizer increases leaf production of mulberry (Sudhakar et al., 2000). Amino acid, organic fertilisers, and biofertilizer can minimize the amount of chemical fertilisation (Abd El-Monem et al., 2008). Single leaf area, number of rooted stolens, total chlorophyll content, strawberry varieties

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increased with spraying of 150 and 00 mg/L of zinc + liquorice root extract at 0, 2 and 4gm/L (Dawood and Mohammed 2010).

Strawberry varieties (Dawood and Mohammed 2010), *Calanthe* plantlets showed increasing in shoot length, fresh weight and dry weight of shoots and roots and also in leaf area when media was supplemented with coconut water (Baque et al. 2011). Natural extracts of garlic and seaweed extraction at highest dilution increased number of leaves, fresh and dry weights of shoot and roots of lettuce and P, K, Ca, Mn, Fe, B, Cu and Zn shoots contents (Hafez et al. 2013 on olive and Trinchera et al. 2014). In addition Darwesh et al. 2015 showed that date palm pollen grain extract at 200 and 400 ppm increases height and leaves numbers of in vitro shoots date palm. The hypothesis of this research was subjected to date palm plantlets in the greenhouse by date palm pollen extract to enhancing growth characteristics.

MATERIALS AND METHODS

This work was done in the greenhouse of Central Laboratory for Research and Development of Date palm during 2014–2015 on the plantlets of date palm

(*Phoenix dactylifera* L.) cv. Bartomouda derived by in vitro technique after acclimatisation stage (3–4 leaves and 20–25 cm in length) to investigate the effect of date palm pollen grain extract and mixture of total amino acids (commercial formula), plantlets have (T0) 2.0 g/l NPK one time/2 weeks, (T1) 600 ppm extract date palm pollen grain, (T2) 800 ppm extract date palm pollen grain, (T3) 3 ml/l total amino acids and (T4) 6 ml/l total amino acids. Amino acids treatments: compounds of amino acids as commercial formula (Parkers) each one litre contains Alanine 2000 mg/l, Aspartic acid 2000 mg/l, Aspergine 15000 mg/l, Biolin 100 mg/l, Cysteine 5000 mg/l, Folic acid 1000 mg/l, Glutamine acid 1500 mg/l, Lysine 10000 mg/l, Methionine 10000 mg/l, Nicotinic acid 1,50,000 mg/l, Pantothenic acid 1,00,000 mg/l, Proline 1500 mg/l, Serine 15000 mg/l, Tyrosine 300 mg/l and Valine 500 mg/l. All treatments were done at one time/2 weeks. Contents of date palm pollen grain is listed in Table 1.

Preparation of palm pollen extracts

Extract was prepared by using ethanol (0.1 g pollen and 10.0 ml ethanol), the solvent was removed from the

Table 1 Contents of date palm pollen extract.

Elements	Chemical composition % g/100 g d.w.	Amino acids %	Fatty acids %				
			Saturated fatty acid				
Carbon C	27.8%	Moisture%	28.8	Isoleucine	1.49	Capric acid	0.46
Nitrogen N	54.1%	Ash%	4.57	Leucine	3.34	Lauric acid	4.82
Magnesium Mg	0.12%	Fibre %	1.37	Lysine	2.95	Myristic acid	13.33
Phosphorus P	0.66%	Fat	20.74	Phenylalanine	1.63	Palmitic acid	34.45
Sulfur S	0.69%	Proteins %	31.11	Threonine	1.72	Stearic acid	2.04
Potassium K	5.5%	Carbohydrates %	13.41	Valine	1.81	Arachidic acid	7.32
Calcium Ca	7.0%	Vitamins		Histidine	1.61	Mono saturated fatty acid	
Zinc Zn	281.0 mg/100	A (IU/100 g)	7708.33	Methionine	0.11	Palmitoleic acid	7.07
						Oleic acid	7.19
Iron Fe	241.0 mg/100	E (IU/100 g)	3030.92	Alanine	2.61	Polyunsaturated fatty acid	
Manganese Mn	284.0 mg/100	C (mg/100 g)	89.09	Arginine	1.61	Linoleic acid	14.24
Sodium Na	0.22 mg/100g			Aspartic acid	3.55	Arachidonic acid	4.57
Boron B	309.4 mg/100g			Glutamic acid	1.74	Eicosapentaenoic acid	0.52
Nickel Ni	302.4 mg/100			Glycine	2.24		
Cobalt Co	305.4 mg/100			Serine	1.89		
Copper Cu	319.6 mg/100 g			Cysteine	0.42		
Molybdenum Mo	302.2 mg/100			Tyrosine	1.55		
				Proline	0.28		

Table 2 Total indole contents of pollen extracts (mg/g pollen)

Pollen extract indole content	
Water extract	10 ± 0.34
Ethanol extract	9 ± 0.21

obtained extract by evaporation. The residue was re dissolved in the same volume of DW (10.0 ml) as given by Nagai et al. 2002.

Vegetative growth determination as: plant height (cm), leaves numbers/plant, fresh and dry weights of leaves (g)

Leaves chemical contents as:

Chlorophyll a and b and carotenoids contents mg/g f.w.: as described by Lichtenthaler and Wellburn (1985).

Total indole mg/g f.w.: as described by Larsen et al. (1962) and Salim et al. (1978)

Amino acids mg/g f.w.: as described by Moore and Stein 1954

Protein contents: were measured as given by Bradford 1976

Carbohydrates: as given by Dubois et al. 1956

Experimental design: Complete randomised design with three replicates and three plantlets for each one, two growth seasons. Data were analysed by analysis of variances (ANOVA) and the means were compared following t-test using LSD values at 5% level (Snedecor and Chocran 1990).

RESULTS

The effect of pollen grain extract and amino acids treatment on all measured characteristics had significant

differences were described. There was a significant increasing effect of pollen grain extract on the plant height (Table 3 and Fig. 1) from 26.7 and 29.3 cm respectively for 1st and 2nd of control treatment to 50.1 and 61.3 cm respectively for 1st and 2nd of 800 ppm pollen grain extract, however two levels of amino acids showed descending order for increasing plant height as mean value 39.2 and 47.9 cm respectively for 3 and 6 ml/l, high interaction was found with level two treatment. Also highest interaction between 800 ppm and pollen grain extract (Table 3 and Fig. 1) showed the greatest significant numbers of leaves arise from 800 ppm 10.3 and 11.3 leaves/plantlet, respectively, for 1st and 2nd followed by 6 ml/l amino acids 9 and 9.3, respectively, for 1st and 2nd leaves/plantlet with significant varies in between compared to control treatment that have mean value 3.3 and 4.7, respectively, for 1st and 2nd leaves/plantlet, 800 ppm date palm pollen grain extract produced highest interaction, significant maximum leaves fresh and dry weight (Table 3 and Fig. 1) come out from 800 ppm of pollen grain extract 22.8 and 24.2 g for fresh weight and 10.8 and 11.6 g respectively for 1st and 2nd. Meanwhile 600 ppm resulted 15.9 and 17.0 for leaves fresh weight and 7.4 and 8.1 g for leaves dry weight respectively for 1st and 2nd, descending by amino acids treatment that increased fresh and dry weights of leaves 15.5 and 16.2 g for leaves fresh weight and 7.1 and 7.7 g for dry weight respectively for 1st and 2nd respectively. Little weights of leaves gave by control treatment 11.4 and 12.2 g and 5.4 and 5.7 g respectively for fresh and dry weights of leaves and 1st and 2nd, significant interaction with highest levels of date palm pollen grains extract on plant height. In respect to the effects of different concentrations of date palm pollen grains extract and amino acids on date palm plantlets leaves chlorophyll a mg/g f.w. contents

Table 3. Effect of different concentrations of date pollen grain extract and amino acids on vegetative growth of date palm at two seasons

Treatments		Plant height (cm)			Leaves numbers/plant			Leaves fresh weight (g)			Leaves dry weight (g)		
		1 st season	2 nd season	Mean	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean
A	Con	26.7	29.3	28.0	3.3	4.7	4.0	11.4	12.2	11.8	5.4	5.7	5.6
Pollen grain ppm	600	41.0	45.0	43.0	7.3	9.3	8.3	15.9	17.0	16.5	7.4	8.1	7.8
	800	50.1	61.3	55.7	10.3	11.3	10.8	22.8	24.2	23.5	10.8	11.6	11.2
	mean	39.3	45.2		7.0	8.4		16.7	17.8		7.9	8.5	
Amino acids ml/l	con	26.7	29.3	28.0	3.3	4.7	4.0	11.4	12.2	11.8	5.4	5.7	5.6
	3	36.7	41.7	39.2	6.6	7.5	7.1	15.5	16.2	15.9	7.1	7.7	7.4
	6	40.3	55.5	47.9	9.0	9.3	9.2	20.1	23.2	21.7	10.1	11.0	10.6
	mean	34.6	42.2		6.3	7.2		15.7	17.2		7.5	8.1	
L.s.d.		A = 1.2 B = 1.5 AB = 2.1 A = 2.9 B = 3.6 AB = 5.1			A = 1.1 B = 1.3 AB = 1.9 A = 1.1 B = 1.3 AB = 1.9			A = 0.8 B = 0.7 AB = 1.0 A = 0.4 B = 0.5 AB = 0.7			A = 0.3 B = 0.4 AB = 0.6 A = 0.2 B = 0.4 AB = 0.4		

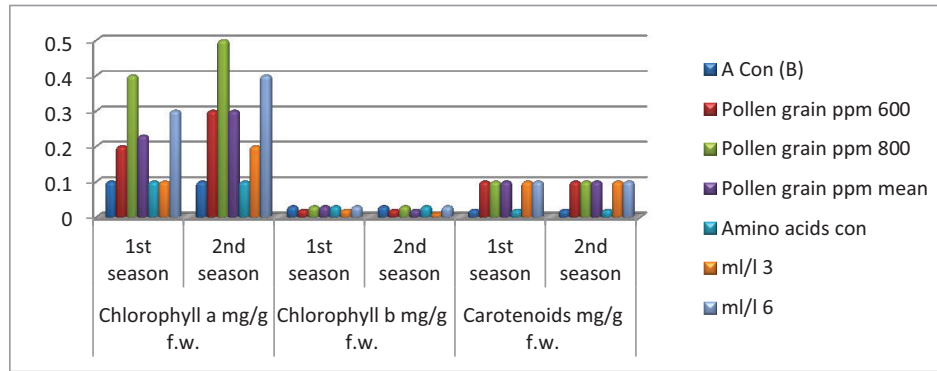


Fig 1 Effect of date palm pollen grain extract and amino acids compounds on chlorophyll two seasons.

(Fig. 1) exhibited significant difference under two treatments and levels. Date palm pollen grains extract 600 and 800 ppm produced 0.2 and 0.3 and 0.4 and 0.5 mg/g f.w. respectively for 1st and 2nd and two levels. Significant little amounts of leaves chlorophyll a occurred with control treatment 0.1 and 0.1 respectively for 1st and 2nd mg/g f.w. with insignificant difference in between. Chlorophyll a contents under amino acids treatments have the second rank 0.1 and 0.2 and 0.3 and 0.4 mg/g f.w. respectively for 1st and 2nd and two levels 3 and 6 ml/l. Level two of pollen grain extracts caused significant interaction, chlorophyll b (Fig 1) showed insignificant varies between 800 ppm of extract of date palm pollen grains and 6 ml/l amino acids and control treatment on the chlorophyll b which produced 0.03 mg/g. High level of two treatment produced significant interaction. Leaves contents of carotenoids (Fig. 1) increased above control treatment 0.02 mg/g f.w. and two treatments gave insignificant results between treatments 0.1 and 0.1 mg/g f.w. respectively for 1st and 2nd. Concerning the leaves contents of indoles mg/g f.w. (Fig. 2) greatest results came out by high levels of two treatments. Lowest contents obtained under control treatment 5.6 and 6.3 mg/g f.w. respectively for 1st and 2nd. Moreover highest indoles contents graduated from 600 ppm pollen grain extract 8.6 and 8.8 mg/g f.w. respectively for 1st and 2nd to 9.6 and 10.9 mg/g f.w. respectively for 1st

and 2nd. Different levels of amino acids have left significant higher contents of indoles than control treatment which confirmed 6.9 and 7.5 mg/g f.w. and 8.6 and 9.6 mg/g f.w. respectively for 1st and 2nd and two levels 3 and 6 ml/l. Carbohydrate contents greatly increased under treatments pollen grain extract of date palm and amino acids compounds (Fig. 2) 800 ppm. Date palm pollen grains led to increasing contents of carbohydrates mg/g d.w. 7.0 and 7.6 mg/g d.w. for 1st and 2nd exceeded by 6 ml/l amino acids compounds 6.5 and 6.9 mg/g d.w. for 1st and 2nd. However 600 ppm extract of pollen grains and 3 ml/l amino acids have a second order under high levels, little accumulated of carbohydrates was obtained by control treatment 2.4 and 4.1 mg/g d.w. 800 ppm pollen grains and 6 ml/l amino acids have high interaction. Results of amino acids (Fig. 3) indicated significant accumulation was found under high levels of two treatments, superiority of increasing to 800 ppm pollen grain extract 3.1 and 3.5 mg/g f.w. respectively for 1st and 2nd, 6 ml/l amino acids compound treatment have left 2.8 and 3.3 mg/g f.w. respectively for 1st and 2nd. However little accumulation obtained with control treatment 1.9 and 2.4 mg/g f.w. respectively for 1st and 2nd, great interaction exists between high levels and two treatments. Protein contents (Fig. 3) revealed significant accumulated ascending from control treatment 3.5 and 3.9 respectively for 1st and 2nd which produced little amount

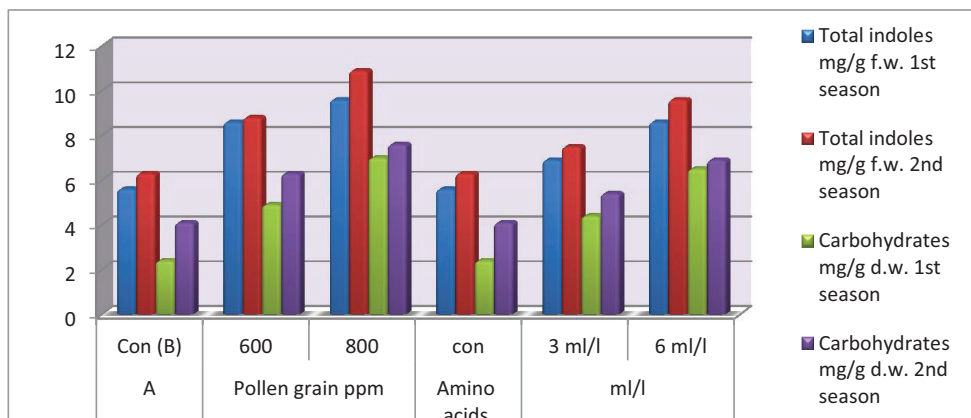
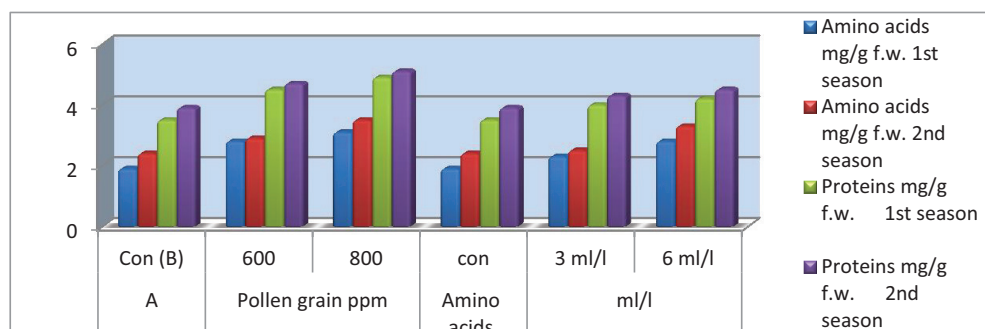


Fig. 2 Effect of date palm pollen grain extract and amino acids compounds on total indoles mg/g f.w. and carbohydrates at two seasons.



Con, 1 = 800 ppm date palm pollen grain 2 = 6 ml amino acids

Pic (1) Effect of date palm pollen grain and total amino acids on vegetative growth of date palm plantlets

Fig. 3 Effect of date palm pollen grain extract and amino acids compounds on amino acids and proteins contents mg/g f.w. at two seasons.

of proteins contents to 600 and 800 ppm extract of date palm pollen grain which confirmed maximum amounts 4.5 and 4.7 and 4.9 and 5.1 mg/g f.w. respectively for 1st and 2nd and two levels with significant differences in between that proceeded by levels of amino acids 4.0 and 4.3 and 4.2 and 4.5 mg/g f.w. respectively for 1st and 2nd and 3 and 6 ml/l levels, major interaction exists between 800 ppm and pollen grain extract treatment.

DISCUSSION

Date palm plantlets under acclimatisation stage in the greenhouse needs different treatments to enhance them in a short time. Current approach in the agriculture management that reduced the chemical fertilisers to decrease the harmful effects to soils and plants also to humans. Crop residues became benefits in protecting environment and reducing pollution, improving soil conditions, minimising water and mineral leaching, also decreasing agricultural costs (Ogbodo 2010). Nowadays approaches emphasise the need to eco-friendly agricultural practices for sustainable agriculture and asset to control environmental pollution (Fawzy et al. 2012 and Dongre and Simon 2013). Date palm pollen grain extract contain different amino acids which have important role in the plant cells and different substances and compounds structure. Free amino acid (AA) released from organic matter can be taken up by roots, soil solution free amino acids contents range increased to 1–10 mM, with glutamic acid (Jones et al. 2005). Organic farming makes more

sustainable production without adverse effects on the natural resources and the environment (Ram et al. 2014). Growth promoting effect of pollen grain extract at 600 and 800 ppm and amino acids 3 and 6 ml/l on the plant height of date palm plantlets, leaves numbers, fresh and dry weight of leaves, furthermore, 800 ppm of pollen grain extract of date palm produced greatest estimation of growth characters, number of shoots, fresh and dry weights of date palm cv Khanezi have increased when 5% date syrup known as 'Dibs' (Al-Khateeb 2008), Aloe vera extract at 140 g/pot as powder or 75% as liquid concentration increased fresh weight of *Abelmoschus esculentus* L and *Oenothera biennis* (Padmaja et al. 2007 and El-Shayeb 2009), date palm plantlets cv. Medjol height, leaves numbers, fresh and dry weights increased significantly with yeast extract at 40 and 50 ml/l, (Rasmia 2010). Soil and foliar protein hydrolysate applications increased growth of agricultural crops e.g, corn, banana, papaya, strawberry, red grape (Gurav and Jadhav 2013). Leaf number/plant of Marigold (*Calendula officinalis* L), shoot dry weight, leaves chlorophyll content, and carbohydrate leaf content increased under extract of sheep manure applied at 40% (Hassan et al 2014). Spraying garlic extract at 8% combined with GA at 100 ppm is recommended to improve productivity and fruit quality of 'Le Conte' pear trees (AbdEl-Razek et al. 2013), improving shoot growth and plant water status of citrus under application of seaweed extract foliar and drench (Spann and Little 2011), enhanced shoot and root length of swamp cabbage (*Impoecaaquatica*) and okra (*Hibiscus esculentus*) when

treated by aqueous extract of bohera (*Terminalia belerica*) leaves (Roy et al. 2012). Kelp extract (1:25 to 1:100) from *Durvillaea potatorum* and *Ascophyllum nodosum* (Seasol Commercial) stimulates significantly leaf area and numbers, stem diameter and length and fresh and dry weights of broccoli also on Pea (Mattner et al. 2015 and Yusuf et al. 2012), increasing bunch mass of *Musa paradisiaca* and plant height with seaweed bio stimulants 4L/ha, also bud height and diameter of bud and stem and leaf area of *Gerbera jamesonii* under Stimplex (a bio-product of *Ascophyllum nodosum*) at 2.5 lit/ha (Adriaan and Errol 2012 and Saeed et al. 2012). Moringa leaf extract at 15 or 25 ml/l or under diluted 1:30 increased plant height, fresh and dry matter *Zea mays*, *Cenchrus ciliaris* and *Panicum antidotale*, *Echinochloa crusgalli* (Culver et al. 2013 and Nouman et al. 2012), plant height increment, leaf number per plant, shoot number per plant, stem diameter, leaf dry weight %, root number, root length of olive (*Olea europaea* L.) increased with algae extract 0.5 litre per bag. Humic acid 20% (Haggag et al. 2014), fenugreek seed extract at 0.5 to 1% was increased at shoot length and leaf area of Keitte mango and total chlorophyll, sugars (Ahmed 2015). Moreover, commercially available amino acid stimulants can improve fertiliser assimilation, increase uptake of nutrients and water, enhance the photosynthetic rate and dry matter partitioning and hence increase crop yield. Spraying of diphenylamine and tryptophan at 50 or 100 ppm significantly increased plant height, number of leaves/plant, stem diameter and fresh and dry weights of *Philodendron erubescens* and Florida prince Peach trees (Abou Dahab and Abd El-Aziz 2006 and Abd El-Razek and Saleh 2013), 5-10% free amino acids increased total soluble sugars, total free amino acids and chlorophyll a and b (El-Kosary et al. 2011 on *Mangifera indica* cvs Keitt and Ewais), number of leaves and fresh weight of date palm cvs Khalas and Sukkariat-Yanbo were significantly increased with 5-aminolevulinic acid from 200 to 250 ppm (Al-Qurashi and Awad 2011 and Awad 2008 on date palm and Aml et al. 2011 on olive seedling (*Olea europaea* L.)), Glutamic acid from 100-200 increased plant height, number of leaves, stem diameter and fresh and dry weights (Azza et al. 2011 on *Cordia alliodora* L.).

Also date palm pollen grains treatments showed the highest effective in achieving a large leaf chemical contents i.e. chlorophyll a, b and carotenoids, indoles, carbohydrates, proteins, and amino acids which closely attributed to increasing plant growth were significantly increased under pollen grain treatment and amino acids, superiority of maximum contents were found with date palm pollen grain extract at 800 ppm. In this respect, dry yeast 4 g/l and NPK at 6 g/l increased chlorophyll and carotenoids and leaves contents of total carbohydrates, nitrogen, and potassium of *Salvia officinalis* L., *Calendula officinalis* (Abd El-Latif 2006, Ahmad and Abdel-Wahid 2007). Highest growth of *Schefflera arboricola*, total carbohydrates given with garlic extract followed by yeast extract, aloe extract and finally henna extract (Hanafy et al. 2012). Humic acid or nicotinamide increased

significantly photosynthetic pigment, total soluble sugar, total carbohydrates, total amino acids and proline and mineral contents N, P, K, Ca and Mg (El-Bassiouny et al. 2014). Date palm plantlets cv. Bartomouda under different amino acids 100, 200 and 300 mg/l or compounds of amino acids at 3 and 6 g/l increased chlorophyll a, b and carotenoids, amino acids, and indole contents (Darwesh et al. 2014 and Darwesh 2013). Chlorophyll a, b of *Arachis hypogea* L., total carbohydrates, and total proteins were increased under 2% seaweed extract (Selvam and Sivakumar 2014). Licorice extract at 4g/L increased growth, TSS, total sugar, of pear *Pyrus communis* x *Pyrus ussuriensis* (Sheren and Eman 2015).

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