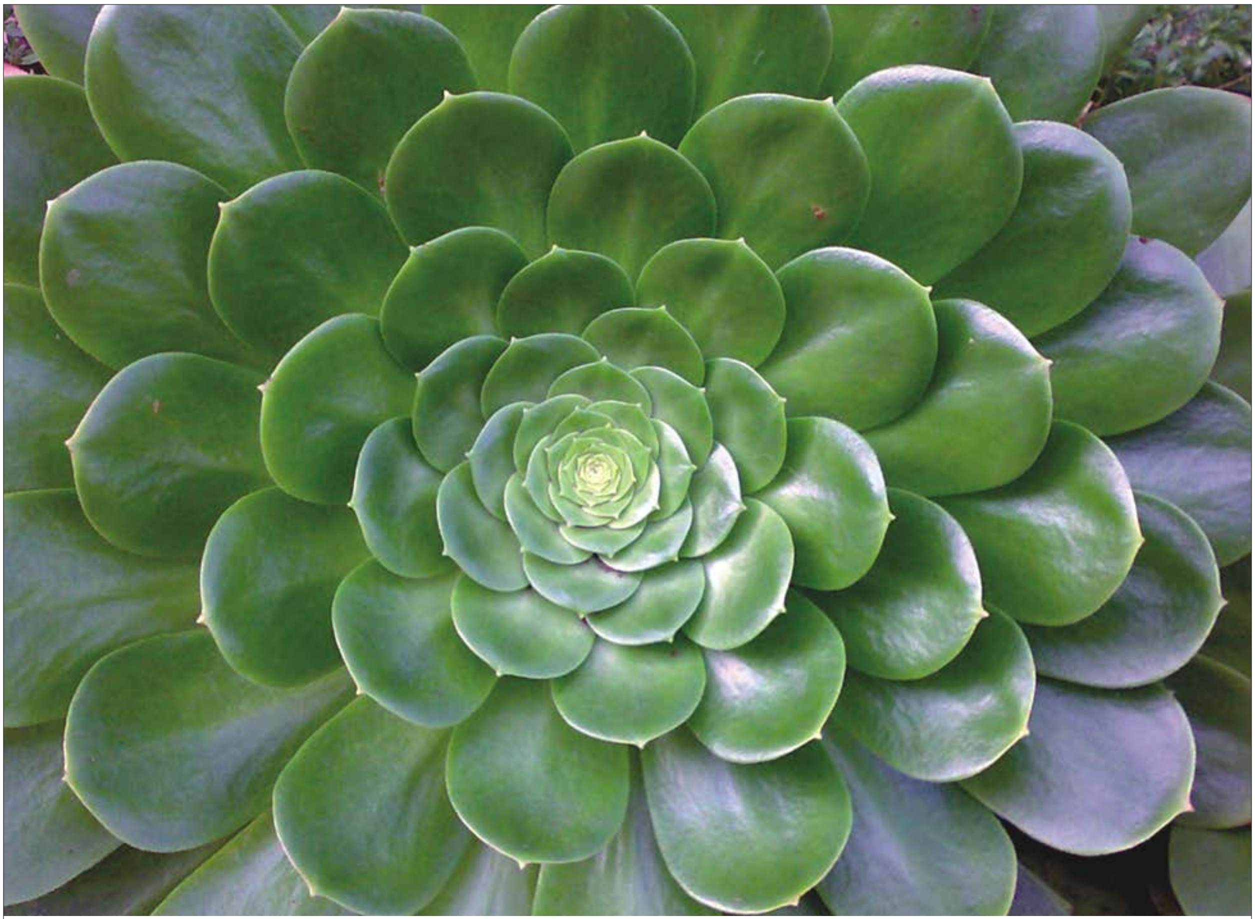

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Research Article
Effect of Nano and Molecular Phosphorus Fertilizers on Growth and Chemical Composition of Baobab (*Adansonia digitata* L.)

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Abstract
Objective: This study was carried out to investigate the effectiveness of foliar application of different sources of P on growth performance of *Adansonia digitata* in Egypt. **Materials and Methods:** Different sources of P [monoammonium phosphate (MAP), diammonium phosphate (DAP) or hydroxyapatite nanoparticles (nHA)] and unfertilized seedlings (control) were used as a foliar application to study growth parameters and chemical composition of *Adansonia digitata* grown in a sandy soil during two successive seasons (2014 and 2015). **Results:** Baobab plants sprayed with nHA showed a significant increase in plant growth characters (plant height, stem diameter, number of leaves per plant, leaf area, root length, total fresh and dry weights) when compared to control plants. Moreover, significant increase in total chlorophyll, carotenoids concentration, total carbohydrates percentage, vitamin C, macro-elements (N, P and K%), crude protein and total phenols content. The anticancer activity of *Adansonia digitata* leaves against Ehrlich Ascites Carcinoma Cells (EACC) and the antioxidant activity of leaves over control plants were recorded in the same treatment. Meanwhile, it had significant reducing power methods. **Conclusion:** It is recommended to spray with nHA for increasing plant growth, nutrition status, DPPH and anticancer activity besides, their safety for either environment or human health.

Key words: *Adansonia digitata*, nanofertilizers, foliar application, FTIR, DPPH, anticancer activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Monkey bread or African baobab tree (*Adansonia digitata* L., Malvaceae) is a deciduous tree that originally located in Africa but can be found in large quantities in America, Malaysia and India (Sidibie and Williams, 2002). It is tolerant to high temperature and long spans of drought (Wickens, 1982). It is culturally and economically important for local populations (Assogbadjc *et al*., 2005).

Baobab is also considered as a multipurpose tree where every part is used daily by rural poor for food because of its nutritive value of the leaves, pulp, seeds and seed oil besides, its use in folk medicine to treat fevers, dysentery, measles, malarial and sores (FAO., 1988; Van Wyk and Gericke, 2000; Masola *et al*., 2009). Furthermore, the addition of baobab pulp powder to temperature fermentation could prevent the growth of pathogenic bacteria such as *Salmonella* sp., *Bacillus* sp. and *Streptococcus* sp. (Afolabi and Popoola, 2005).

For achieving a significant increase in *Adansonia digitata* production many factors are involved such as evaluation of the best source of nutrients, suitable timing and proper fertilizer optimum rates of fertilization (FAO., 2005).

Phosphorus (P) is classified as a 2nd most important major nutrient next to nitrogen (N) in limiting crop growth because it is one of the 17 essential nutrients required for plant growth (Raghothama, 1999). It has a vital role in respiration, photosynthesis, biosynthesis, nucleic acids, energy generation and as an integral component of several plant structures such as phospholipids (Vance *et al*., 2003).

Despite its importance in plant growth, P is the least accessible macronutrient and available for plant uptake in the most agricultural tropical soils, mainly because of its low availability, its fixation with Ca in alkaline soils and its poor recovery from the applied fertilizers (Marschner, 1995). In addition, the reserves of global P are being depleted at a high rate and according to some estimation by the year 2050; there will be no soil P reserve (Cordell *et al*., 2011).

Hydroxyapatite particles, HA ($Ca_{10}(PO_4)6(OH)_2$) nanoparticles are ranked as one of the most popular candidates in agronomic applications which can provide the phosphorus nutrients. Much of the present study on HA is focused on its biomedical applications due to its excellent biocompatibility and bioactivity (Venkatasubbu *et al*., 2013) while, potential cultivated and applications have not been adequately resolved.

The foliar method of fertilizer application is usually preferred because very small amounts of fertilizers are applied per hectare. In addition, it decreases the number of passes of the applicant thereby, reducing a problem of soil compactness. Foliar application is one of the ways to increase production in sustainable agriculture and as well less likely to result in ground water pollution and was found most effective and economical to improve the plant growth (Ali *et al*., 2008; Hamayun *et al*., 2011).

As a result of the unavailability of phosphorus in Egyptian soil and because of its importance as one of the essential macronutrient for plant growth for this reason, the objectives of this study were to study the effectiveness of foliar application of different sources of P on the growth parameters, chemical composition as well as antioxidant and anticancer of *Adansonia digitata* grown in a sandy soil for increasing accessible macronutrient (P) and to be available for plant in Egypt.

MATERIALS AND METHODS

A pot experiment was carried out in 2014-2015 at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and studies, Cairo University, Giza, Egypt. The aim of this study was to investigate the effect of different sources of phosphorus foliar application to improve vegetative accrementition, chemical composition of baobab seedlings which led to increase its numbers for use it in various fields especially in the medical one in economic way in Egypt.

Plant material: Fruits of *Adansonia digitata* L. (Labill.) were obtained from certain mother trees, Agriculture station of El-Khartoum, Sudan. Seeds from cracked dry fruits were soaked in a boiling water for 5-7 min then left to cool overnight (Diop *et al*., 2016). On 1st May the pre-treated seeds were sown in plastic pots, 25 cm diameter, filled with a sandy sterile soil having the following characteristics: Coarse sand 30.82%, fine sand 62.61%, silt 1.22%, clay 5.35%, pH 7.75, EC 1.15 dS m^{-1}, organic matter 0.08%, available N 6.9 (ppm), available P 6.2 (ppm), available K 64 (ppm), $CaCO_3$ 0.26% and water holding capacity 14.5%.

Synthesis and characterization of hydroxyapatite nanoparticles: Hydroxyapatite particles were prepared by co-precipitation of calcium and phosphate solutions. The morphology and size of the particles were examined by TEM under careful conditions with the use of co-precipitation of solution mixtures of calcium chloride and phosphoric acid followed by centrifugation and calcination. Rod-shape hydroxyapatite nanoparticles were obtained.

Hydroxyapatite nanoparticles were synthesized by Mateus *et al.* (2007) using aqueous solutions of calcium hydroxide [$Ca(OH)_2$] and orthophosphoric acid (H_3PO_4) 85%. In this experimentation Ca:P molar ratio was sustained at 1.67. First, 0.6 M H_3PO_4 (250 mL) and was added drop-wise into a suspension of calcium hydroxide (19.29 g $Ca(OH)_2$ in 250 mL water) while, stirring vigorously under mechanical agitation (1000 rpm).

The HA nanoparticles (nHA) were allowed to precipitate and the supernatant was poured. The resulting HA nanoparticles were twice washed with a distilled water. The obtained solid was thus dried at 100°C for 2 h. The product was characterized using transmission electron microscopy (TEM, JEOL 2010F) and fourier transform infrared spectroscopy (FTIR, JASCO FTIR spectrometer, FT/IR-4600).

Phosphorus fertilization treatments: Foliar application of 20 mL of different sources of P as monoammonium phosphate (MAP), diammonium phosphate (DAP) and hydroxyapatite nanoparticles (nHA) was sprayed onto the leaves of plants (25 cm tall) per pot weekly. Unfertilized seedlings were used as a control.

Experimental design: The experimental study was carried out in 2014-2015 based on Randomized Complete Block Design (RCBD) including three treatments plus control in three replicates, with each block consisting of 20 plants (five plants per treatment).

At the end of the experiment (1st Septamber in the two seasons, respectively) the following data were recorded.

Growth parameters: Plant height (cm), stem diameter (cm), number of leaves per plant, root length (cm) total fresh and dry weights gram per plant. Leaf area (cm^2) was also measured with area meter.

Chemical analysis

Photosynthetic pigments, total carbohydrates and vitamin C: Fresh leaves were extracted with 5 mL of dimethylformamid (DMF) for overnight at 5°C to measure total chlorophyll and carotenoids according to Moran (1982). Total carbohydrates (%) in the dried leaves were also determined by using 5 mL of 67% sulphuric acid as described by DuBois *et al.* (1956). Vitamin C as ascorbic acid (mg g^{-1} fresh weight) was determined and estimated per 100 mL fresh leave juice according to AOAC (1990) method.

Determination of macro-elements and crude protein: Dried leaf samples were digested and the extract was analyzed to determine nitrogen (N%) using the modified Micro-Kjeldahl method, phosphorus (%) due to Jackson (1967) while, K% was determined by a flame spectrophotometer (Al-Khayri, 2002). The nitrogen percentage was multiplied by 6.25 to estimate the crude protein percentage as described by James (1995).

Preparation of ethanol extract of *Adansonia digitata* L.: The dried *Adansonia digitata* L., leaves (100 g) were ground into powder and extracted with 100 mL ethanol 70% at 37°C overnight. The extraction was repeated twice, the total extracts were filtered and the obtained filtrates were concentrated under vacuum using rotary evaporator to dryness. The crude extracts were stored at -20°C until use.

Measurement of total soluble phenols: The total phenols concentration of four ethanolic extracts was determined with the folin-ciocalteu reagent (Singleton *et al.*,1999). A standard curve was plotted using different concentrations of gallic acid and the result was expressed as gallic acid (μg mL^{-1}) in equivalent.

Measurement of DPPH free-radical scavenging activity: Quantitative measurement of radical scavenging properties was determined by the method of Blois (1958). Discoloration was measured at 517 nm after incubation for 30 and 60 min. Measurements were performed atleast in triplicate. The actual decrease in absorption induced by the test compounds was compared with the positive controls.

Reducing power: The reducing power of all ethanol extracts was determined as described by Dorman *et al.* (2003) and after that the absorbance of the solution was measured spectrophotometrically at 562 nm. The value was expressed as Gallic Acid Equivalent (GAE).

Viability of Ehrlich Ascites Carcinoma Cells (EACC): Viability of EACC which were incubated with various extracts of baobab and saline solution as control was determined by the modified cytotoxic trypan blue-exclusion technique as described by Bennett *et al.* (1976). For each examined material (control), 10 μL of cell suspension, 80 μL saline solution and 10 μL trypan blue (0.4%) were added and mixed then the number of living cells (non-stained) was calculated using a homocytometer slide by microscope (Nikon, TMS).

The obtained data were subjected to statistical analysis of variance and the means were compared using the "Least Significant Difference (LSD)" test at the 5% level as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Characterization of photo-catalysts by TEM: The hydroxyapatite nanoparticles shape and size are presented in Fig. 1a and b. The particles are rod shaped with dimension of 59.5×10.6 nm under high magnification HRTEM.

FTIR: The FTIR spectrum as displayed in Fig. 2 showed the characteristic absorption peaks of HAP sample. Theoretically, there were four vibrational modes present for phosphate ions, v_1, v_2, v_3 and v_4. The analysis revealed the presence of the v_1 and v_3 phosphate bands in the region 900-1 and 200 cm^{-1} and v_4 absorption bands in the region 500-700 cm^{-1} which characterized the structure of apatite. The spectral band in the range 900-1 and 200 cm^{-1} containing the symmetric v_1 and asymmetric v_3, P-O stretching modes of phosphate groups at 1,095 and 1,041 cm^{-1} were observed for the prepared powder. The symmetric P-O stretching mode for HA occurs at 953 cm^{-1} and this was confirmed by Hongquan *et al.* (2003).

The FTIR spectrum of the prepared hydroxyapatite is illustrated in Fig. 2. It is well known that carbonate ion can substitute both hydroxyl ion and phosphate ion in hydroxyapatite. The peak corresponding to the OH^- stretching mode at 3,568 cm^{-1} can be easily shown. Moreover, bands

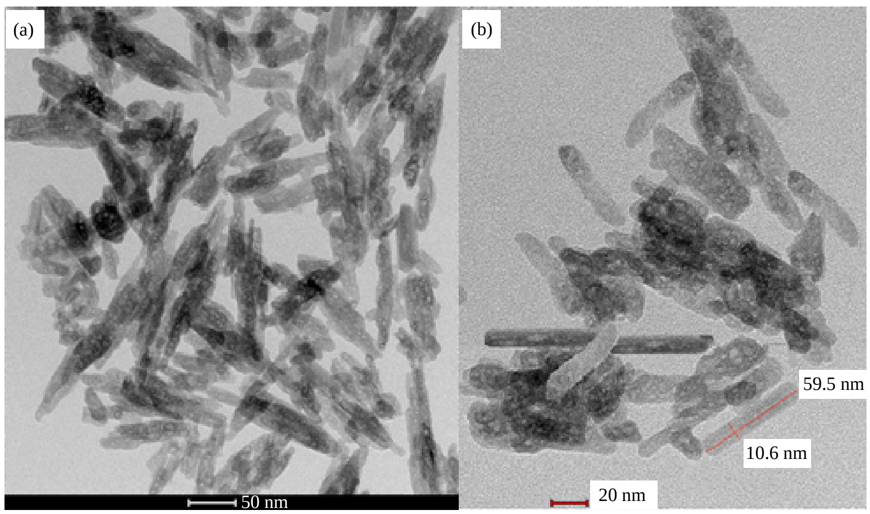

Fig. 1(a-b): (a) TEM image of hydroxyapatite and (b) High-magnification HRTEM image of hydroxyapatite nanoparticles

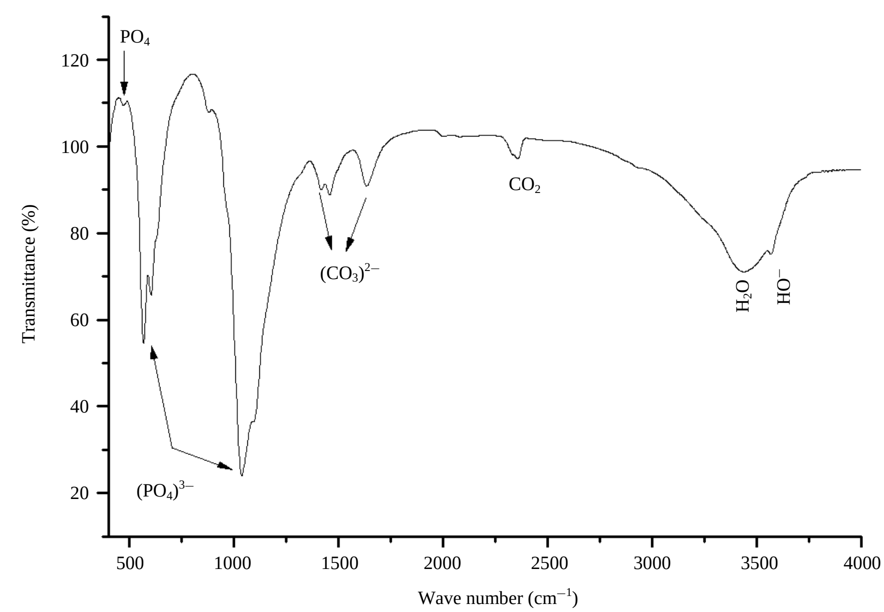

Fig. 2: FTIR spectra of hydroxyapatite synthesized by $Ca(OH)_2$ and H_3PO_4

corresponding to the carbonate ion can be also shown which suggest that the powder may have a carbonate-substituted apatite structure. The bands at 1,041, 953, 601 and 568 cm^{-1} can be assigned as members of the anti-asymmetric bending motion of the phosphate group on HAP. Furthermore, there were bands at 1,419, 1,457 and 1,636 cm^{-1} which were assigned to the carbonate group (CO_3^{-4}). The FTIR results are similar to those reported by Murugan and Ramakrishna (2004) and Nejati *et al.* (2009).

FTIR study: The functional groups of plant dry leaves were identified by using FTIR spectroscopy between the scan ranges of 400-4000 cm^{-1}. The obtained FTIR spectrum for *Adansonia* plank sample, treated sample and synthesized HA are shown in Fig. 3a-c. Figure 3a displays a number of absorption peaks such as 3425 cm^{-1} assigned for OH of alcohols/phenols, 2925 cm^{-1} for C-H stretch of alkanes, 1740-1630 cm^{-1} for C=O of carboxylic acids, ketones, aldehydes and esters stretches, 1425 cm^{-1} for OH bend of carboxylic acids, 1316 cm^{-1} for C-H bond of alkenes, 1027 cm^{-1} for HCOO bond of carboxylic acids, 774 cm^{-1} for C-H bond of aromatic benzene and 654 cm^{-1} for C-H deformation of alkynes reflecting its complicated nature. The peak at 580 was assigned as a rocking vibration of CO_2^- and r (CO_2^-).

The peaks at 1035 and 560 cm^{-1} for PO_4^{-3} groups in HA (Fig. 3c) disappeared as shown in Fig. 3b for nHA/*Adansonia* reatment which may be due to the composite formation. The peak intensity at 1027, 1630 and 3425 cm^{-1} in control was decreased after composite formation (Fig. 3b) which evidences that the COOH/OH groups from *Adansonia* chemical components have taken a part in bonding with hydroxyapatite PO_4^{-3} groups. Most of the peaks in nHA treated *Adansonia* showed a shift after composite formation. Thus, a comparison of the FTIR analysis indicated that there is a chemical bonding between the HA nanoparticles and *Adansonia* carboxylic acids. Figure 4 illustrates the proposed ester linkage formed between hydroxyapatite PO_4^{-3} and *Adansonia* (COOH/OH groups).

Effect of different sources of P on growth parameters: The results of analysis for the growth parameters (plant height, stem diameter, number of leaves per plant, root length total fresh and dry weights and leaf area) are presented in Table 1. Growth parameters were highly significant responded to nHA treatment followed by the secondary DAP plants and primary orthophosphate ions MDP then control plants. Total dry weight (gram per plant) was increased by 35.1 and 30.5% in the 1st and 2nd season, respectively although, these increments were in the favor of control plants which recorded 11.57 and 9.43 g $plant^{-1}$, respectively. Preceding results are in harmony with those obtained by Vance *et al.* (2003) who reported that plant dry weight may contain upto 0.5% phosphorus. These increases in growth parameters may be attributed to the beneficial effects of nanoparticles which have high reactivity because of more specific surface area, more density of reactive areas or increased reactivity of these areas on the particle surfaces. These features simplify the absorption of fertilizers that was produced in nano scale (Anonymous, 2009). Moreover, nHA led to stimulate the growth of the root zone allowing the plant to get nutrients and water to distant areas of metabolic activity (Uarrota, 2010). Furthermore, increases of cytokinins synthesis (Horgan and Wareing, 1980). The findings from this study are consistent as indicated by Tisdale and Nelson (1975), Fageria (2009) and

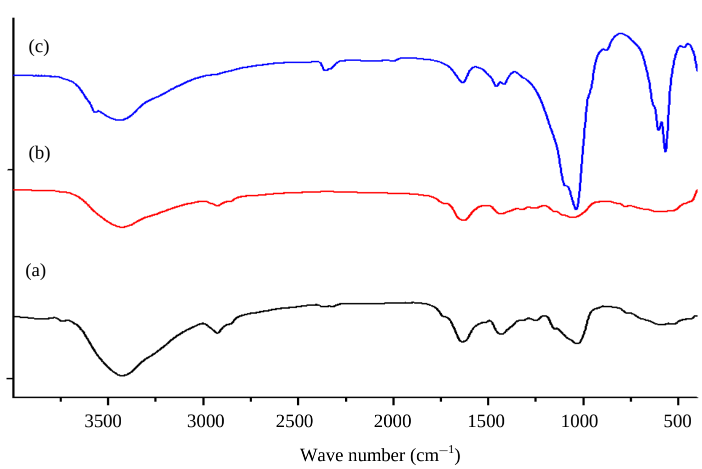

Fig. 3(a-c): IR spectra for deride leaves of *Adansonia digitata* grown under different treatments of phosphorus, (a) Control (b) The nHA treated plant and (c) The nHA

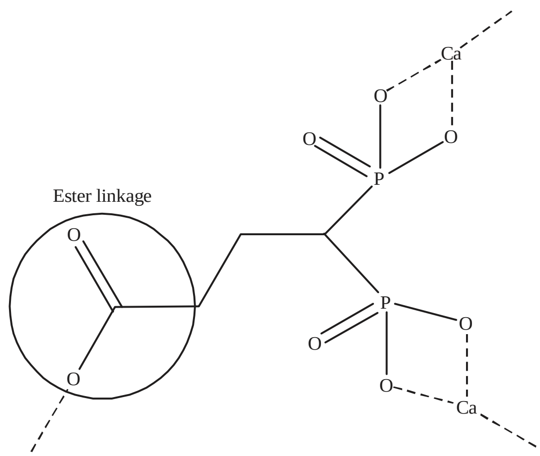

Fig. 4: Schematic representation of ester linkage formed between hydroxyapatite phosphate groups and carboxylic acids in the *Adansonia* chemical components

Table 1: Effect of different sources of P on growth parameters of *Adansonia digitata* during two seasons 2014 and 2015

Treatments	Seasons	Plant height (cm)	Stem diameter (cm)	No. of leaves	Leaf area (cm^2)	Root length (cm)	Total fresh weight (g $plant^{-1}$)	Total dry weight (g $plant^{-1}$)
Control	1st	35.00	2.00	19.00	18.97	27.00	43.50	11.57
	2nd	31.33	1.70	16.00	17.83	21.33	38.46	9.43
MAP	1st	42.00	3.50	22.33	25.33	33.33	69.64	19.89
	2nd	37.67	2.90	18.67	21.37	29.33	59.93	13.81
DAP	1st	55.00	5.20	29.00	36.63	45.00	87.37	25.91
	2nd	48.67	3.80	26.33	28.68	37.67	85.76	21.36
nHA	1st	60.67	6.00	37.00	45.20	52.67	98.21	33.00
	2nd	55.00	4.87	32.33	41.60	46.67	93.00	30.93
LSD (0.05)	1st	13.46	2.31	8.26	9.49	2.69	16.43	2.02
	2nd	9.49	1.92	1.88	1.97	2.18	4.61	10.16

MAP: Monoammonium phosphate, DAP: Diammonium phosphate and nHA: Hydroxyapatite nanoparticles

Table 2: Effect of different sources of P on chemical composition of *Adansonia digitata* during two seasons 2014 and 2015

Treatments	Seasons	Total chlorophyll (mg g^{-1} fresh weight)	Carotenoids (mg g^{-1} fresh weight)	Total carbohydrates (%)	N (%)	P (%)	K (%)	Crude protein (%)	Ascorbic acid (mg g^{-1} fresh weight)
Control	1st	1.24	0.60	31.33	1.9	0.22	1.52	11.88	1.03
	2nd	1.06	0.47	29.00	1.87	0.19	1.48	11.69	0.70
MAP	1st	1.47	0.71	33.00	2.19	0.38	2.57	13.69	1.77
	2nd	1.23	0.60	32.33	2.11	0.33	2.43	13.19	1.10
DAP	1st	2.22	1.08	36.33	2.25	0.41	2.89	14.06	2.33
	2nd	2.08	1.01	34.67	2.19	0.39	2.71	13.71	1.83
nHA	1st	2.58	1.23	37.67	2.46	0.58	3.03	15.38	2.83
	2nd	2.49	1.16	35.00	2.39	0.52	2.90	14.94	2.47
LSD (0.05)	1st	0.06	0.22	6.07	0.16	0.14	0.20	0.96	0.81
	2nd	0.13	0.13	4.95	0.83	0.89	0.17	3.37	0.76

MAP: Monoammonium phosphate, DAP: Diammonium phosphate and nHA: Hydroxyapatite nanoparticles

Uarrota (2010). In addition, supportive evidence for this view was reported by Onasanya *et al*. (2009) on maize and Liu and Lal (2014) on *Glycine max*.

Effect of different sources of P on chemical composition: The nHA treatment significantly affected the chemical composition (total chlorophyll, carotenoids concentration, vitamin C, total carbohydrates, N, P, K% and crude protein) of *Adansoina digitata* as compared to control plants (no fertilizer application) in both seasons (Table 2). These augmentations with reference to total chlorophyll, carotenoids concentration, total carbohydrates, crude protein and ascorbic acid in baobab seedlings were 45.4, 44.8, 62.2, 77.7 and 32.6%, respectively over control plants. Meantime, the increment of main macro-elements N, P and K were 77.7, 37.3 and 50.5%, respectively over control plants. The success of nHA as

Table 3: Anticancer, antioxidant activities and total phenols of *Adansonia digitata* L., extracts

		Antioxidant activity			
		DPPH (inhibition percentage)			
Extract	Total phenols as GAE equivalent (μg mL^{-1})	30 (min)	60 (min)	Reducing power as GAE	Anticancer dead cells (%)
Control	133±3.0	63±1.0	72±1	144±0.0	0
MAP	143±3.0	59±0.0	66±0.0	138±12	81.33±3.06
DAP	145±0.0	61±1.0	67±0.0	133±17	81.76±4.51
nHA	145±0.0	85±0.0	87±0.0	143±2	75.00±2.0
BHT		85±2.0	95±2.0		

MAP: Monoammonium phosphate, DAP: Diammonium phosphate, nHA: Hydroxyapatite nanoparticles and BHT: Standard antioxidants butylated hydroxytoluene

fertilizer may be due to a greater density in reactive areas which increased the uptake of phosphorus and that eventaually led to enhance in leaf moisture percentage, total chlorophyll, crude protein, total carbohydrates and plant nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur percentage (Trivedy *et al*., 2003; Dhiraj and Kumar, 2012; El-Kereti *et al*., 2013). In addition, Ehiagiator *et al*. (2011) concluded that higher application of P may expose the plant nutrient to larger surface area that can enhance fixation of the nutrient. Positive interaction is also known to occur between phosphorus and the other nutrients such as nitrogen, sulphur and zinc (Kang and Juo, 1979). The aforementioned data are in trustworthiness with those of Raj *et al*. (2003) on mulberry, Sawan *et al*. (2008) on *Gossypium barbadense* and Khosa *et al*. (2011) on *Gerbera jamesonii*:

$$\text{Total phenols } (Y = 0.002X+0.004 \text{ at } R^2 = 0.887)$$

Table 3 recorded the total phenols of ethanol extracts of *Adansonia digitata* L., total phenols in the control, MAP, DAP and extracts were computed to be 133±3, 143±3 and 145±0.0 μg mL^{-1} as GAE, respectively.

DPPH radical scavenging activity: The values of percent DPPH scavenging activities of *Adansonia digitata* ethanol extract were summarized in Table 3 as comparable with known antioxidant such as BHT. In most cases as shown in Table 3, the highest percent DPPH radical scavenging activity was observed in nano fertilizer treatment which gave 87±0.0% followed by control treatment 72±1%, MAP and DAP which showed lower scavenging activity (66±0.0 and 67±0.0%, respectively) at 60 min incubation. These results show that compounds with the strongest radical scavenging activity in *Adansonia digitata* exhibited time-dependent to DPPH radical scavenging activity. All tested extracts had lower antioxidant activity than the BHT which gave 95±2% at 60 min. These results suggested that ethanol extracts of *Adansonia digitata* (nano group) are good scavengers for DPPH radical.

Reducing power: The reducing power of the tested ethanol extracts of *Adansonia digitata* L. was determined as gallic acid from the standard curve with the linear equation Y = 0.003x at R^2 = 0.728. It was evident that the nanofertilers extract had a higher reducing power than the ethanol extracts of normal fertilizers (Table 3).

Anticancer activity against Ehrlich ascities carcinoma cells: In the present study, the values of percent anticancer activities of *Adansonia digitata* were summarized in Table 3. The potent anticancer activity was observed in all tested ethanol extracts which gave 81.33±3.06, 81.76±4.51 and 75±2.0 in MAP, DAP and nHA%, respectively. These results suggested that these extracts were good anticancer agents. There is significant difference between nHA tested group and control group.

The elevated amount in percent anticancer activities in *Adansonia digitata* plants treated with nHA foliar application may be attributed to increase the uptake of phosphorus that affect in direct or indirect ways in increasing a variety of chemicals from *A. digitata* such as terpenoids, flavonoids, steroids, polyphenolic compounds, vitamins, amino acids, carbohydrates and lipids (Shukla *et al*., 2001; Brady, 2011; Nordeide *et al*., 1996; Scheuring *et al*., 1999) and in increasing also, antioxidant (vitamin C and carotenoids) which shows their therapeutic potential (Kaur and Kapoor, 2001; Besco *et al*., 2007; Lamien-Meda *et al*., 2008; Chadare *et al*., 2008; Brady, 2011) such as inflammation, cardiovascular disease, cancer and aging-related disorders as they may eliminate free radicals which contribute to these chronic diseases (Willet, 2001; Carlsen *et al*., 2010). From these data nHA foliar application led to improve the growth of *Adansonia digitata* plants grown in soil suffer from lack of availability of phosphorus in Egypt to use it as remedial natural products in the treatment of cancer and nourishment. Furthermore, using natural products are not bringing side effects on opposite of malignancy medications are bringing side effects in some instances (Abdel-Hady *et al*., 2011).

CONCLUSION

Hydroxyapatite nanoparticles (nHA) foliar application played a critical role in the significant increase phosphorus availability to the plant which led to improving growth parameters, chemical compositions, good scavengers for DPPH radical and anticancer activity of *Adansonia digitata* especially, under new area (sandy soil) compared to control plants (no fertilizer application) and that will be reflected on increasing the quality and quantity of baobab plants in Egypt and making it available for use in various fields especially in the medical field in economic way.

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