

# Evaluation of the productivity of *Cyperus esculentus* L. cultivated in different phytogeographical locations in Egypt (*ex-situ* conservation) and its anti-oxidant activity

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## Background and objectives

The conservation and management of threatened and endangered species is a tremendous challenge that must be addressed to achieve the goal of halting the loss of plant biodiversity. According to the IUCN Red List of Threatened Species, *Cyperus esculentus* lies in the least concern category. This means that it has been evaluated against the Red List criteria and does not qualify for critically endangered, vulnerable, or near threatened. In Egypt, the people depend mainly on the wild growing tubers, whereas the area of land cultivated with tiger nut is becoming very small. This is highly affecting the population of the plant and explains the need to introduce its cultivation in different phytogeographical regions of Egypt.

## Materials and methods

Tubers of *C. esculentus* were obtained from Wady Elsheh Farm, Assuit Governorate, Egypt, and were cultivated in four different locations during two planting periods. Physical and chemical properties of the soils were determined. In addition, water used for irrigation was analyzed. Moreover, growth and yield parameters were recorded. The 80% methanol extract of powdered tubers was prepared using the ultrasound-assisted extraction, and the antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay.

## Results and conclusion

The obtained results showed that soil type and water used for irrigation had a significant effect on plant growth, that is, plant height and fresh and dry weight of herb during the two seasons.

Moreover, the four extracts of samples obtained from the four locations exerted remarkable antioxidant activity (83.4, 92.4, 90.7, and 65.1%, respectively), which could be attributed to high flavonoids content of the tubers.

The soil and water for irrigation at Wadi Elsaieida, Aswan Governorate, and El-Bahrya Oasis are more suitable for the production of *C. esculentus* tubers.

## Keywords:

antioxidant, *Cyperus esculentus*, conservation, Cyperaceae, 2, 2-diphenyl-1-picrylhydrazyl, endangered species, Hab El-Aziz, phytogeographical regions, tiger nuts

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## Introduction

*Cyperus esculentus* L. belongs to the family Cyperaceae, which is known to be rich in flavonoids, sesquiterpenes, alkaloids, quinines, and coumarins [1]. Sesquiterpenes isolated from the tubers and rhizomes exhibited bioactivities such as insecticidal, anti-malarial, anti-fungal, anti-bacterial, and anti-inflammatory activity [2]. *C. esculentus* is a sedge plant, which has other names such as yellow tiger sedge, chufa, and earth-almond [1] and is known in Arabic as Hab El-Aziz. In Egypt, it was used as an important source of food [3], medicine, and perfumes [4]. Tiger nut is a crop of early domestication and was added to other crops of the Nile Valley; its dry tubers have been found in tombs from predynastic times ~6000 years ago [5].

Historically, the cultivation and utilization of tiger nut tubers have started with the Egyptians at ~5000 BC [6,7]. Since then, tiger nut has spread to other parts of the world. Tiger nut is one of the wild edible plants that could be used to improve human nutrition. The tubers and roots of the plant are used to help in respiratory, ailments, cough, and heat and sharpness of urine [3,8]. Tubers are also useful in eye diseases, burning sensation, and leprosy [9]. Some people chew the roots to relieve indigestion, especially when the condition is accompanied by bad breath. The

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primary purpose of cultivating tiger nut in Egypt is because of the sweet tubers, but many people who eat the tubers are not well informed about their benefits. This is one of the reasons tiger nut is still regarded as being underutilized.

The medicinal benefits of tiger nuts are ascribed to a large number of nutritional components it contains. Sugar-free tiger nut milk is suitable for diabetic patients [10]. They are thought to be beneficial to those seeking to reduce cholesterol or lose weight [11]. It is good for arteriosclerosis [12]. According to Stern *et al.* [13], Paiute Indians pound tiger nut tubers with tobacco leaves applied en masse on wet dressing for treatment of athlete's foot. The 80% alcoholic extract of defatted powder of *C. esculentus* tubers showed hepatoprotective activity [14].

In Egypt, *C. esculentus* is endangered and/or under threat of extinction. The conservation and management of threatened and endangered species is a tremendous challenge that must be addressed to achieve the goal of halting the loss of plant's biodiversity. For this reason, we started a project on ex-situ conservation and genetic enhancement of *C. esculentus* tubers. Ex-situ conservation is the process of protecting or preserving the plants outside of its natural habitat by placing in a new environment which may be a wild area or within human control environment.

In this work, the plant was cultivated in four different phytogeographical regions. Physicochemical analysis of soils was done. In addition, irrigation water was analyzed. The growth parameters and plant yield were recorded. The antioxidant activity was determined for the prepared extracts from different locations.

## Materials and methods

### Locations

This investigation was carried out during two successive seasons (2016/2017 and 2017/2018), to evaluate the growth and productivity and of *Cyperus esculentus* grown in four different locations in Egypt.

The locations were:

- (1) El-Minia Governorate
  - (a) Sekem Farm: It lies about 450 km south of Cairo, 40 kilometers from the city of Minya and 10 kilometers from the Nile River. Coordinates of farm location 27°58'34.98" North, 30°54'47.88" East.
  - (b) Saft El Khamar

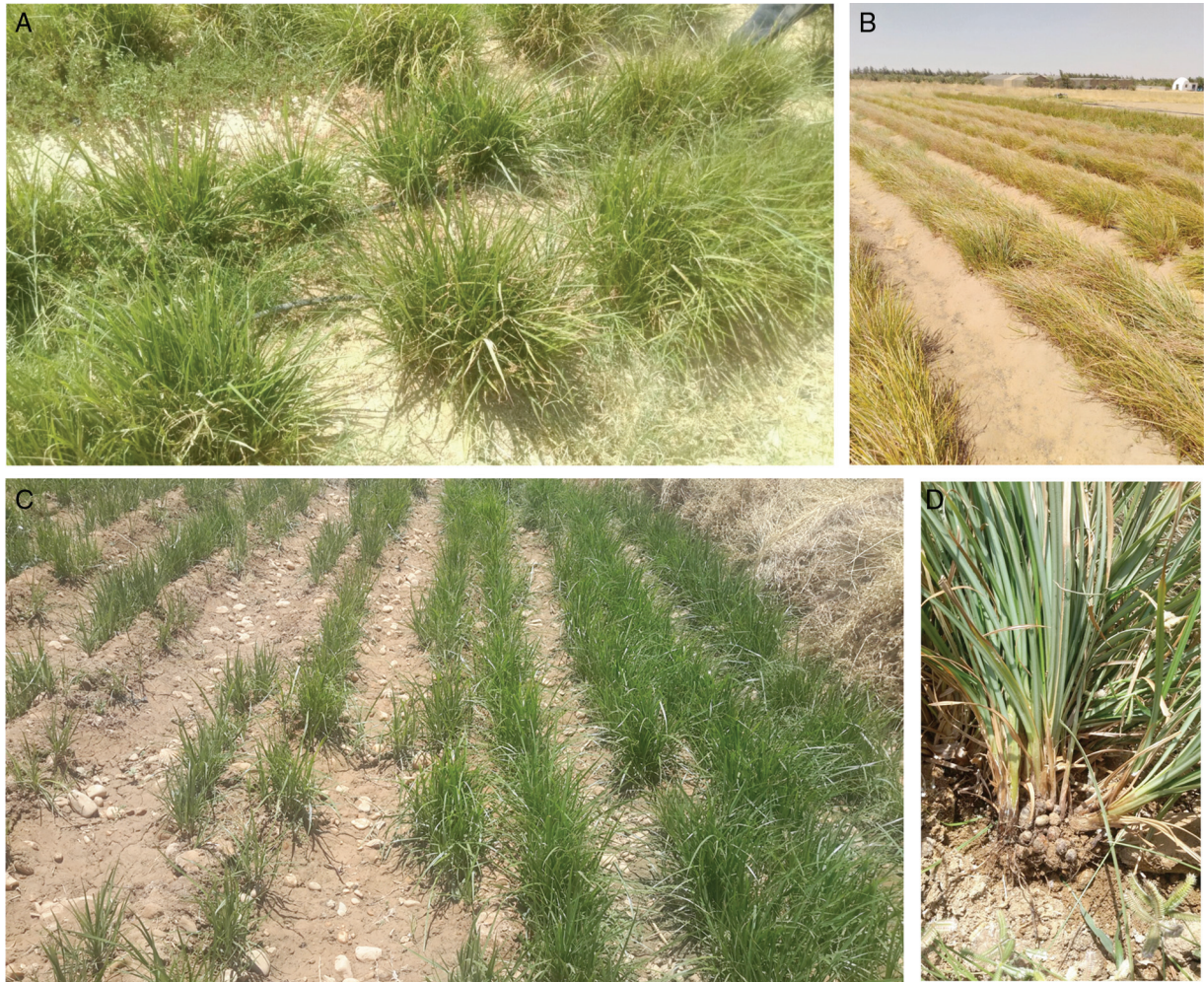
- (c) Nearby cities: El Minya, Abu Qirgas, Samalut
  - (d) Coordinates: 28°1'47"N 30°41'33"E
  - (e) Saft El Khamar is a village belonging to Minya, Minya Governorate.
- (2) Bahariya Oasis: It is located at the west of the Nile Valley about 180 Km (West of Menya). Within 270 48, and 280 30/ latitude to the north and 280 35/ and 290 10/ longitude to the east.
    - (a) Sekem Field: SEKEM bought new farmland 346 acres (333 feddan) in Al-Hara village at Bahariya Oasis, which is 25 km from Bawiti, about 350 Km away from Cairo. Al Bawīṭī is situated at 28.35° North latitude, 28.87° East longitude and 142 meters elevation above the sea level. Al Bawīṭī is a small city in Egypt, having about 20,000 inhabitants.
  - (3) Aswan Governorate:
    - (a) Wadi al-Sa'aida is a village belonging to the Edfu Center in Aswan Governorate, Egypt.
  - (4) El- Sharkia Governorate:
    - (a) The Experimental Station of Medicinal and Aromatic Plants of El-Adlya Farm, Sekem Company, Belbis, EL-Sharkiya Governorate.

### Cultivation practices

Tubers of *C. esculentus* were obtained from Wady Elsheh Farm, Assuit Governorate, Egypt, and cultivated in four different locations (Fig. 1). The tuber transplants were placed at 5 cm apart from each other, with rows 30 cm apart. Plants were cultivated (45 kg/fed) in 25 March 2017 and 2018. Plants were irrigated immediately after transplanting and later as required to maintain vigorous growth. The physical and chemical analyses of the soil and water used for irrigation characters were conducted according to Jackson [15], and Cottenie *et al.* [16]. The results are shown in Tables 1 and 2. In different locations, vegetative characteristics of the plants were determined after 6 months from sowing. The irrigation for the area in all location was between 3 and 7 days according to soil types and environmental conditions at each location. Fertilization per feddan was added at a rate 200 kg/feddan super phosphate 15.5% P<sub>2</sub>O<sub>5</sub> and 100 kg/feddan ammonium sulfate 20.6 N in preparing land for sowing, and after 45 days from sowing, added 100 kg ammonium nitrate 33.5% per feddan, and now the plants can be seen in a vegetative stage in all locations according to the attached photos for *C. esculentus* in fields. The harvest processes were conducted by hand in November 2017 and 2018, and the growth parameters, viz. plant height (cm), root length (cm), tubers' number, tubers' fresh weight (g/plant), tubers' dry weight (g/plant), herb fresh weight (g/plant), herb



Figure 1



Tubers of *Cyperus esculentus* cultivated in different locations: (a) Minya Sekam Farm. (b) Wahat Baharia. (c) Aswan Wady Elsayda. (d) El-Sharkia.

dry weight (g/plant), root fresh weight (g/plant), and root dry weight (g/plant), were recorded (Table 3).

#### Extraction process

Four samples of tiger nut tubers (*C. esculentus* L.) grown in the two planting periods (March 2017 and 2018) were studied. Eight extract solutions were prepared from dried *C. esculentus* L. tubers. The 80% methanol extracts of different grinded tubers were done using the ultrasound-assisted extraction. The ultrasound-assisted extraction technique condition was used according to Hassanein and colleagues, using Ultrasonic Processor UP400S (400 W, 24 kHz, Hielscher), with 100% amplitude, 0.5 cycles, 300 ml solvent for each 50-g plant and was held for 30 min. The alcohol extracts were then filtered and dried at 45°C under pressure using rotavapor device, where a yield of 30.6% was obtained from the total dry weight used.

#### 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 20 µl (4 mg/ml) of

extract diluted appropriately in DMSO was mixed with 180 µl of DPPH in methanol (0.4 mg/ml) in wells of a 96-well plate. The plate was kept in the dark for 15 min, after which the absorbance of the solution was measured at 540 nm in a Multiskan automatic kinetic microplate reader (Labsystems Multiskan RC reader). Appropriate blanks (DMSO) and standard (trolox solutions in DMSO) were run simultaneously. Extracts were first tested at a single concentration of 2 mg/ml. This method follows closely that used by previous authors [17].

#### Statistical analysis

All recorded data were subjected to analysis of variance procedures, and treatment means were compared using least significant difference at 5% [18], as described by Snedecor and Cochran. All data were subjected to analysis of variance and significant means were compared with Duncan multiple range test method, performed using SPSS package (Statistical Package for

the Social Sciences). Statistical program, version 19. IBM company program..

## Results

Soil and water analyses data are shown in Tables 1 and 2. Data presented in Tables 3 and 4 indicate that different locations had a significant effect on growth and yield characteristics during the two seasons. In the first season (2017) (Table 3), Wadi Elsaicida, Aswan Governorate, gave the highest mean values of plant height (27.12 cm) and dry and fresh weights of tubers (20.83 and 55.21 g/plant, respectively). On the contrary, plants

cultivated under El-Baharyia Oasis location conditions produced the maximum mean values of root length (22.82), number of tubers (20.33/plant), dry and fresh weight of roots (22.41 and 114.30 g/plant, respectively) as well as dry and fresh weight of herb (34.73 and 364.7 g/plant, respectively).

In the second season (Table 4), plants cultivated under El-Bahrya Oasis gave the maximum mean values of plant height (27.40 cm), root length (19.44 cm), tubers number (21.12/plant), root dry weight (19.36 g/plant), root fresh weight (96.34 g/plant), herb dry weight (32.90 g/plant), and herb fresh weight (345.64 g/plant). Moreover, plants cultivated

**Table 1 Chemical and physical properties of the studied soils**

Locations	Chemical soil properties		Soluble cations and anions (meq/l)							
	E.C 1 : 5 (Ds/m)	pH (2.5 : 1)	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>--</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>--</sup>
El-Menia Sekem	7.69	1.14	3.40	2.40	5.10	0.80	–	1.40	7.50	2.80
Aswan Wadi Al-Saaida	8.00	0.56	1.00	0.50	3.60	0.90	–	1.90	3.50	0.60
El-Sharkia	1.11	7.85	2.38	1.10	6.11	16	–	279.0	1.48	0.25
El-Baharya Sekem	7.78	0.43	0.70	0.30	2.60	0.50	–	1.10	2.00	1.00
Soil texture and its fractions										
Locations	Sand %		Silt %		Clay %		Texture class			
El-Menia Sekem	75		17.9		6.5		Sandy loam			
Aswan Wadi Al-Saaida	72.4		19.0		8.6		Sandy loam			
El-Sharkia (El-Adlya)	81.5		7.4		11.1		Sandy clay loam			
El-Baharya Sekem	69.3		10.2		22.1		Clay loamy sand			

**Table 2 Chemical properties of water irrigation for studied locations**

Locations	EC 1 : 5 (Ds/m)	PH (2.5 : 1)	Soluble cations (meq/l)				Soluble anions (meq/l)			
			Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>--</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>--</sup>
El-Menia										
Sekem Farm	0.96	7.82	4.0	1.2	3.6	0.87	–	0.9	2.80	5.97
Aswan										
Wadi Al-Saaida	0.69	8.08	2.1	0.80	3.80	0.30	–	1.50	5.0	0.5
Bahariya Oasis										
Sekem Farm	0.38	7.14	1.10	0.5	1.8	0.50	–	1.3	2.3	0.5
El-Sharkia										
El-Adlya Farm	1.59	7.77	1.00	1.00	15.00	0.144	–	2.80	6.50	7.80

**Table 3 Effect of different locations on growth and yield characteristics of *Cyperus esculentus* plants (season 2017)**

Locations	Plant height	Root length	Tubers number	Tubers dry weight	Tubers fresh weight	Root dry weight	Root fresh weight	Herb dry weight	Herb fresh weight
Minya Sekem Farm	16.94c	7.50d	9.11b	9.52c	23.81c	6.11b	30.56d	19.01d	171.1d
Aswan Wadi Elsaicida	27.12a	13.52b	19.33a	20.83a	55.2a	15.59ab	84.18b	25.28b	240.14b
El-Bahrya Oasis	25.69a	22.82a	20.33a	12.91b	32.92b	22.41a	114.30a	34.73a	364.70a
Sharkia (Aladlya Farm)	19.57b	10.92c	9.00b	7.03d	18.43d	9.61	46.13c	22.73c	209.66c
LSD at 5%	1.91	1.34	2.08	3.07e-7	2.00	7.39	2.31	2.00	10.99

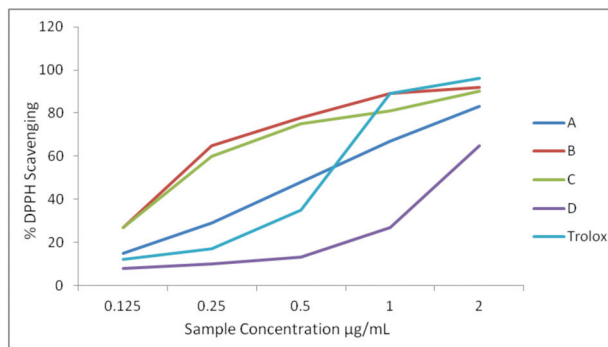
LSD, least significant difference. a) El-Minia (Sekem), b) Bahariya Oasis (Sekem), c) Aswan Wadi al-Sa'aida d) El- Sharkia of *C. esculentus* tuber.



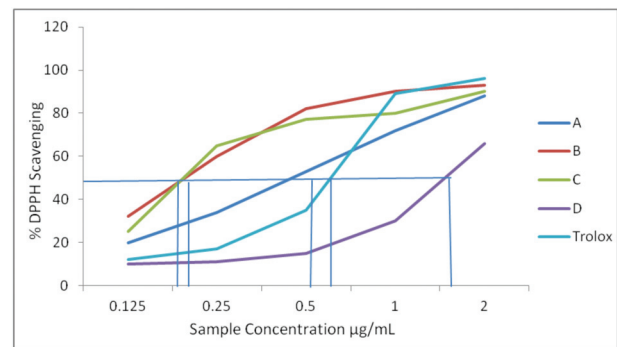
**Table 4** Effect of different locations on growth and yield characteristics of *Cyperus esculentus* plants (season 2018)

Locations	Plant height	Root length	Tubers number	Tubers dry weight	Tubers fresh weight	Root dry weight	Root fresh weight	Herb dry weight	Herb fresh weight
Minya Sekem Farm	17.26c	8.12c	9.55c	10,81c	26.9c	7.32d	32.5d	21.6c	184.2c
Aswan Wadi Elsaïda	27.13a	15.76b	14,82b	21.65a	54.22a	16.4b	75.18b	27.34b	255.6b
El-Bahrya Oasis	27.4a	19.44a	21.12a	14.02b	35.17b	19.36a	96.34a	32.9a	345.64a
Sharkia (Aladly Farm)	20.13b	11.02c	8,65c	8.09d	21.62d	10.75c	44.5c	20.16c	189.6c
LSD at 5%	2.51	3.41	1.73	1.63	2.38	2.08	1.91	2.00	13.70

LSD, least significant difference. a) El-Minia (Sekem), b) Bahariya Oasis (Sekem), c) Aswan Wadi al-Sa'aïda, d) El- Sharkia of *C. esculentus* tuber

**Figure 2**

Concentration–response curves for the DPPH radical scavenging activity of trolox (positive control), are A) El-Minia (Sekem), B) Bahariya Oasis (Sekem), C) Aswan Wadi al-Sa'aïda and D) El-Sharkia of *C. esculentus* tuber cultivated in 2017. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

**Figure 3**

Concentration–response curves for the DPPH radical scavenging activity of trolox (positive control), are A) El-Minia (Sekem), B) Bahariya Oasis (Sekem), C) Aswan Wadi al-Sa'aïda and D) El-Sharkia of *C. esculentus* tuber cultivated in 2018. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

under Aswan location produced the highest fresh and dry weights of tubers (54.22 and 21.65 g/plant, respectively) followed by El-Bahrya Oasis, which recorded 35.17 and 14.02 g/plant, respectively.

In general, the superiority of Aswan and El-Bahrya Oasis locations for production of tubers yield may be owing to that the environmental conditions for both locations especially Aswan (soil, water irrigation, and climatic conditions) are suitable for this plant.

#### 2,2-diphenyl-1-picrylhydrazyl assay

From the DPPH assay, all the extracts exhibited antioxidant activity, when compared with the oxidative potential of the standard compound (Trolox, 96.2%) used in this study. The four extracts from each season were studied. Each location showed similar antioxidant when the two seasons are compared together. Samples obtained from Aswan and El-Bahrya Oasis exerted remarkable activity [92.4 and 90.7%, respectively, in 2017 (Fig. 2) and 93.6 and 90.2%, respectively, in 2018 (Fig. 3)]. This could be

attributed to flavonoid content of tubers such as rutin and diosmin [19,20].

#### Discussion

*C. esculentus* L. tubers are rich in polyphenols such as flavonoids, phenolic acids, and other phenolic compounds [18]. Moreover, tubers have high oil percentage, which showed high antioxidant activity [12]. Those facts explain the high antioxidant activity, which was shown in Aswan and El-Bahrya Oasis samples. Moreover, the superiority of Aswan and El-Bahrya Oasis locations for production of tubers yield may be owing to that the environmental conditions for both locations, especially Aswan (soil, water irrigation, and climatic conditions), which are suitable for this plant.

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#### Conflicts of interest

There are no conflicts of interest.

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