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Quantification of Crude Extracts from *Khaya senegalensis* Leaves Irrigated with Agricultural Drainage Water and Treated with Seaweed and some Biofertilizers

Esraa A. M. Mohamed*, Ahmed M. El-Baha, Ahmed A. A. El-Settawy, Nader D. Shetta, Mohamed Z. M. Salem

Forestry and Wood Technology Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt
* Corresponding author: *Esraa.admahmoud@alexu.edu.eg*

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ABSTRACT

In the present work, air-dried leaves from *Khaya senegalensis* seedlings that was irrigated with different irrigation schedules using agricultural drainage water and fertilized with seaweed extract, certain biological fertilizers, and mineral fertilizers-as well as their combinations on the quantity of crude extracts. The crude extracts were carried out using the ethanol solvent and expressed as g/100 g of air-dried leaves. Regarding the irrigation regime's influence, the irrigation that uses 50% Agricultural Drainage Water (ADW) and 50% Nile Water (NW) has produced the highest amount of leaf extract (8.558 g/100 g of air-dried leaves). The seedlings that were fertilized with Biofertilizer (30 ml/bag) containing strains (TS)® (Bio-enriched fluid, Bio Tech Company) comprising *Bacillus circulans*, *Bacillus polymyxa*, *Bacillus megaterium*, *Candida* spp., and *Trichoderma* spp. showed the highest amount of leaf extract, measuring 9.123 g/100 g of air-dried leaves. This indicates the impact of fertilization. The seedlings irrigated with 50% NW + 50% ADW and fertilized with TS 30 ml/bag showed the highest LE (10.567 g/100 g of air-dried leaves) about the significant interaction between the effects of the irrigation regime and fertilization. These seedlings were followed by those irrigated with 100% ADW + without fertilizer (10.372 g/100 g of air-dried leaves).

INTRODUCTION

Khaya senegalensis (Family Meliaceae) is often referred to as Senegal mahogany, Gambia mahogany, Senegal mahogany, dry zone mahogany, and African mahogany. *K. senegalensis* is used as a popular traditional medicine in Africa with its bioactive compounds khayanolides, khayanolide E and two other B, D-seco-limonoids, khayalactol and 1-O-acetylkhayanolide B (Drijfhout & Inwood, 2010).

One of the most important problems in the arid and semi-arid regions is the lack of irrigation water to satisfy cultivated areas in Egypt. The search for another source of irrigation with low quality such as drainage water used alone or mixed with Nile water became a great demand. Furthermore, when water is taken up by the plants, the majority of the salts that are added to the irrigation water are retained in the soil. These salts may build up and make less soil water available for plants (Ehlig, 1960). It should be emphasized that the plant responses to salinity depend not only on irrigation water quality but also on irrigation management (Bernstein & Bramer, 1967). Also, saline water irrigation is considered detrimental to most plants even those salt tolerant, which show a decrease in yield when grown under saline conditions (Ayers & Westcot, 1977).

The use of drainage or saline water becomes necessary for irrigation in new reclaimed area especially in the northern part of Egypt, because of the shortage of Nile water yield in the last years. The suitability of any water irrigation is governed by many factors, such as salt tolerance or plants, type and concentrations of salts in the water and the content of some heavy metals, as well as, on the soil properties (Ayers & Westcot, 1976).

Concerning the changes in chemical properties of the soil due to the quality of irrigation water (Ayers & Westcot, 1985). It was concluded that the chemical properties of soils became worse as a result of using drainage water (El-Tarawy et al., 1991). Furthermore, even though agricultural drainage water contains heavy metal pollutants due to illogical human practices, many farmers reuse it straight without treating it. Chemicals in water, solid wastes, gasses, and smoke-related particulate matter are commonly blamed for pollution. Non-material forms of pollution include excessive light or noise (Nofal, 1981).

There was a policy in place to reuse drainage water for irrigation during the 1970s, according to reports. Reusing drainage water became crucial to water management in the late 1980s due to the scarcity of irrigation water, following several studies conducted by the Drainage Research Institute and other authorities (Bazaraa, 2002). Reusing water was said to be made possible by the vast network of subsurface and open drains that were being built. Egypt is the nation in dry regions that has made the greatest progress in recycling drainage water **(**Soltan, 1988**)**. In Upper Egypt, drainage water is reused by gravity flow. Reusing water in Lower Egypt involves raising it to the irrigation canals. To put the reuse policy into practice, the MWRI Mechanical and Electrical Department (MED) oversaw the construction of 25 centralized mixing stations on the main Nile Delta drains. The 1999 Water Policy Reform Program predicted that by 2017, reuse would reach 8.4 billion cubic meters annually.

In addition to the increasing water demand from the household and industrial sectors, this water will be needed to support the need for new horizontal expansion projects, including those that are already underway. By 1997–2017, the national policy aims to raise the amount of water available by 20%. A fifth of this amount would need to come from greater reuse. Regarding the maintenance of water quality, agriculture needs to be considered a pervasive non-point source of pollution. Leached salts, insecticides, and minerals like nitrogen are examples of pollutants.

To improve the growth, productive yield, biochemical compounds, and nutritional composition of ornamental plants, shrubs, and trees, biostimulants such as seaweed extracts and microbial fertilizations can be applied (Castiglione et al., 2021; Hassanein et al., 2021; Kularathne et al., 2021; Aremu et al., 2022; Kisvarga et al., 2022; Matthews et al., 2022). Researchers have been interested in the biofertilization of horticultural crops, which has emerged as a promising substitute for chemical fertilizers in recent years. In comparison to chemical fertilizers that include nitrogen, biofertilizers pose a comparatively lower environmental risk.

Therefore, the aim of this work was to evaluate the effectiveness of seaweed extract, bio and NPK fertilizers with different irrigation regimes on the amount of extracts from *Khaya senegalensis* leaves.

MATERIALS AND METHODS

Plant collection (Source of plant material)

Fresh leaves samples of *Khaya senegalensis* were collected from a greenhouse experiment in Nubaria city (West of Alexandria), Behira governorate (N30^{O}08̀55” E30^{O}08’42”) from August 1^{st,} 2021 till the end of July 2023. Different irrigation schedules using agricultural drainage water were used throughout the experiment, and the effects of seaweed extract fertilizer, certain biological fertilizers, and mineral fertilizers—as well as their combinations as shown in Table (1) on the quantities of crude extracts.

Preparation of leaf extracts

Fresh *Khaya senegalensis* leaves from each treatment sample were cleaned with tap water, let to air dry at room temperature, and then finely ground in a mixer grinder, then kept until required. The process of making the organic extract (ethanol) from finely ground leaves involved weighing a sample of finely ground leaves and putting it in the appropriate amount of ethanol solvent (15 g of powdered leaves in 100 ml of 70% ethanol) for three days while shaking it occasionally. The extracted material was then filtered through Whatman filter paper No. 1. The solvent was then separated and put into Petri dishes to finish drying before being concentrated using a rotary evaporator (El-Hefny et al., 2022; Elbanoby et al., 2024) (Figure 1).

Table 1: Water irrigation and fertilization regimes and treatment compositions

Treatment		Composition
Three irrigation regimes	W1	100% Nile water (NW) (control)
	W2	50% (NW) + 50% Agricultural drainage water (ADW)
	W3	100% (ADW)
Treatments of fertilizers		
F1		Unfertilized plants (control)
F2		Mineral- fertilized plants (1 g N, 0.5 g P_2O_5, 0.5 g K_2O/bag) (NPK)
F3		Plant applied with seaweed extract (SE) at 7 ml/L (wild brown seaweed *Sargassum* (LIRON500® organic-enriched fluid, Bigchem Company), by w/v consists of lignin sulfonic acid 20%, Zn-EDTA 5% and Mn-EDTA 5%).
F4		Bio fertilized plants (30 ml/bag) with strains (TS)® (Bio-enriched fluid, Bio Tech Company) consists of *Bacillus circulans*, *Bacillus polymyxa, Bacillus megaterium, Candida* spp., and *Trichoderma* spp.).
F5		NPK + TS
F6		NPK + SWE
F7		TS + SWE
F8		NPK + TS + SWE

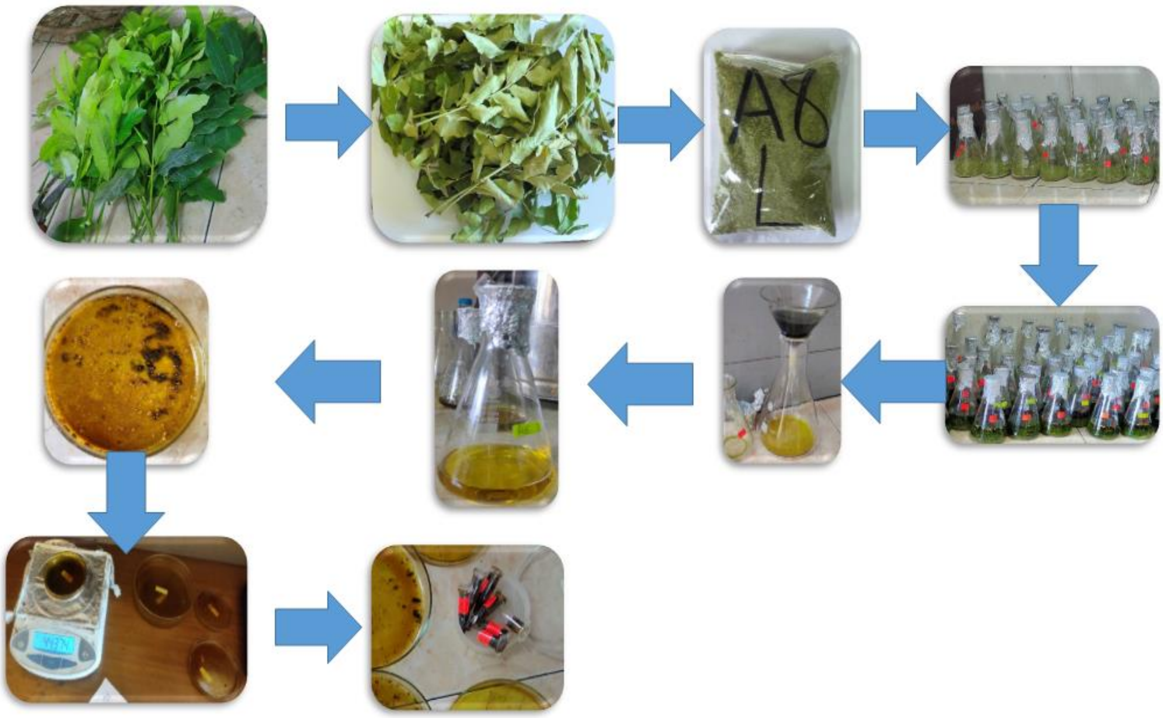

Figure 1: Scheme summarizing the extraction process of crude extracts

Quantitative of crude extracts

The dried yield percentage of crude extracts were weighted to record the quantities using the following formula;

$$Yield\% = \frac{Final\ weight\ of\ extract}{Total\ weight\ of\ milled\ leaves} \times 100$$

Statistical analysis

Data of the crude extracts from the leaves as affected by the irrigation and fertilization regimes were statistically analyzed using the split-plot experiment, where the main plot was for irrigation and the subplots for fertilization (Snedecor & Cochran, 1989).

RESULTS AND DISCUSSION

Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*

The statistical analysis of variance has revealed significant differences among irrigation regimes and fertilization levels in terms of quantity of leaf extract (LE). In addition, there are significant interactions between the irrigation regime and fertilization levels (Table 2).

As for the influence of the irrigation regime, it has been found that the irrigation with 50% Nile water (NW) + 50% agricultural drainage water (ADW) has induced the highest quantity of leaf extract (8.55 g/100 g of air-dried leaves), whereas the lowest quantity of leaf extracts values of 7.35 g/100 g of air-dried leaves, and 7.26 g/100 g of air-dried leaves were obtained in the seedlings irrigated with ADW and NW, respectively (Table 3 and Figure 2).

Concerning the impact of the fertilization, the seedlings fertilized with TS, NPK + Seaweed extract (SWE) 7ml/L, SW 7ml/L, and TS 30ml/ bag + SW 7ml/L displayed the highest quantity of leaf extract with values of 9.12, 8.42, 8.28, and 8.013 g/100 g of air-dried leaves, respectively. In contrast, the lowest LE (6.47 g/100 g of air-dried leaves) was found in seedlings fertilized with NPK + TS 30ml/bag (Table 4 and Figure 3).

Table 2: Analysis of variance (ANOVA) of Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis* irrigated with W_1, W_2, and W_3 and fertilized with F_1, F_2, F_3, F_4, F_5, F_6, F_7, and F_8

S.O.V.	DF.	MS of Quantity of leaf extract
Replication	2	2.8570
Irrigation (A)	2	12.4963*
Rep*Irrigation	4	1.3829
Fertilizer (B)	7	6.8823*
A*B	14	6.7710*
Exp. Error	42	2.5672

Table 3: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with three irrigation regimes

Irrigation regime	Quantity of leaf extract (g/100 g of air-dried leaves)
W1	7.265 b*
W2	8.558 a
W3	7.356 b
LSD 0.05	0.942

*: Means with same letter are not significantly different according to LSD at 0.05 level of probability.

W_1: 100% Nile water (NW).

W_2: 50% (NW) + 50% Agricultural Drainage Water (ADW).

W_3: 100% (ADW).

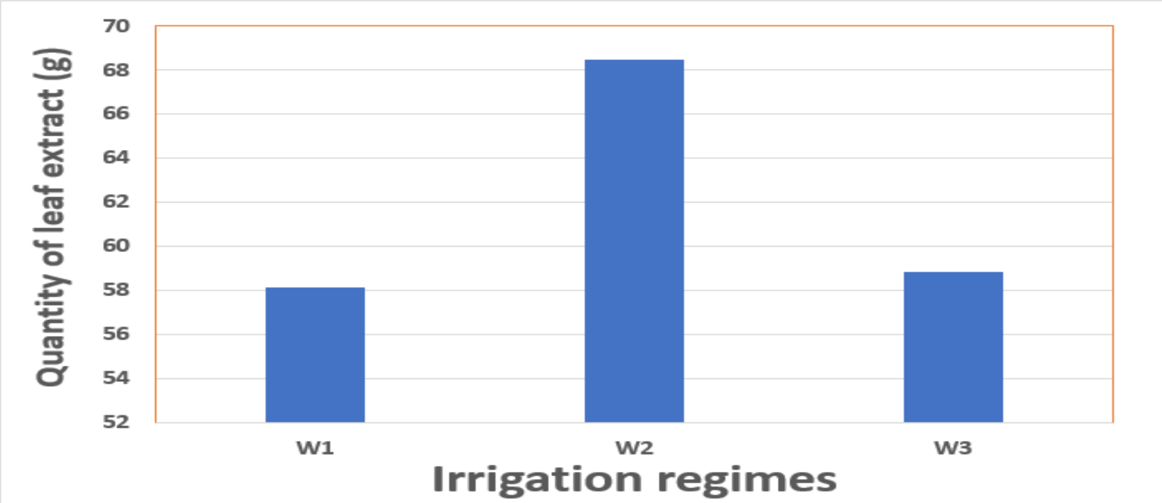

Fig. 2: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with three irrigation regimes. W1: 100%Nile water (NW). W2: 50% (NW) + 50% Agricultural Drainage Water (ADW). W3: 100% Agricultural Drainage Water (ADW)

Table 4: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with eight fertilizer levels

Fertilizer Levels	Quantity of leaf extract (g/100 g of air-dried leaves)
F1	7.206 bc*
F2	7.234 bc
F3	8.281 ab
F4	9.123 a
F5	6.477 c
F6	8.423 ab
F7	8.013 ab
F8	7.054 bc
LSD 0.05	1.524

*: Means with same letter/s are not significantly different according to LSD at 0.05 level of probability.

F_1: Without fertilizer.

F_2: 1g N, 0.5g P_2O_5 and 0.5g K_2O/bag.

F_3: Seaweed extract (SWE) 7ml/L (Organic fertilization).

F_4: Top Strains of Biotechnology (TS) 30ml/ bag (Bio fertilization).

F_5: Mineral fertilization + Bio fertilization.

F_6: Mineral fertilization + Organic fertilization

F_7: Bio fertilization + Organic fertilization.

F_8: Mineral fertilization + Bio fertilization + Organic fertilization.

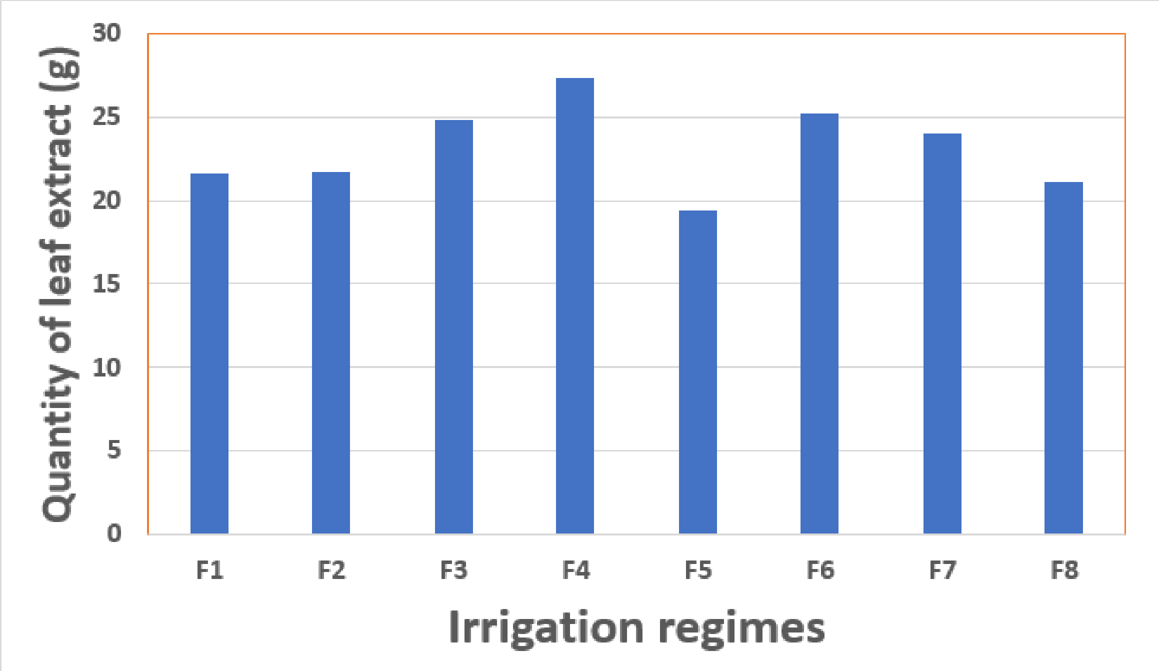

Fig. 3: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with eight fertilizer levels. F1: Without fertilizer. F2: 1g N, 0.5g P_2O_5 and 0.5g K_2O/bag. F3: Seaweed extract (SWE) 7ml/L (Organic fertilization). F4: Top Strains of Biotechnology (TS) 30ml/bag (Bio fertilization). F5: Mineral fertilization + Bio fertilization. F6: Mineral fertilization + Organic fertilization. F7: Bio fertilization + Organic fertilization. F8: Mineral fertilization + Bio fertilization + Organic fertilization.

Regarding the significant interaction between the impact of the irrigation regime and fertilization, the seedlings irrigated with 50% NW + 50% ADW, which were fertilized with TS 30ml/ bag, displayed the highest LE (10.56 g/100 g of air-dried leaves), followed by 100% ADW + without fertilizer (10.37 g/100 g of air-dried leaves), irrigated with 50% NW + 50% ADW and fertilized with SWE 7ml/L (10.05 g), and irrigated with 50% NW + 50% ADW and fertilized with NPK + TS + SWE (9.27 g/100 g of air-dried leaves). In comparison, the lowest values (4.48 g/100 g of air-dried leaves) were detected in the unfertilized seedlings irrigated with NW (Table 5 and Figure 4).

From the above results, it can be found that the fertilizer types and irrigation regimes have a potential impact on the quantities of the crude extracts from *Khaya senegalensis* leaves.

When seaweed extract (SWE) with and without 2 g/L were applied as foliar fertilizer, the plants' essential oil content increased when compared to either 100% chicken manure or 100% inorganic fertilizer with SWE. This was observed when the plants were fed at 50 and 100% of the recommended rate (Eisa, 2016). The impacts of biofertilization and plant extracts were studied, along with the growth, yield, chemical composition, and oil production of basil plants. The results showed that most of the treatments increased the plant's height, branches, dry weight, and oil yield. The most efficient ratio of garlic to yeast was 50% garlic plus 8 g yeast/L (Ahmad et al., 2014). The effects of different amounts of complete fertilizer on the yield, fresh and dry weight, essential oil + composition, total phenolic content, and antioxidant activity of *Satureja hortensis* L. were investigated. The results showed that using fertilizer improved the output and effectiveness of essential oils. The levels of several components, such as carvacrol, γ-terpinene, and α-terpinene, altered when fertilizer was applied (Alizadeh et al., 2010). The application of 100% farmyard manure with SWE outperformed 100% chicken manure or 100% inorganic fertilizer with seaweed extracts in terms of fresh and dry weight, plant height, number of leaves, production of bulbs and fruits, and essential oil. The study also found that the amount of SWE sprayed on plants significantly affected each plant's content, yield per feddan, and proportion of essential oils (Eisa, 2016). The usage of SWE raised the amount of flavonoids and overall phenolic contents in fenugreek. The increase in flavonoid content in SWE-treated plants was more than twice as high compared to the control (Mafakheri & Asghari, 2018).

Growth-promoting hormones (IAA and IBA, Cytokinins), vitamins, amino acids, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), and total phenolic, flavonoid, and tannin levels in leaves are all present in SWE. As a result, SWE boosts the antioxidant activity of plant leaf extracts (Krajnc et al., 2012). Additionally, SWE increases plants' antioxidant capacities (Eris et al., 1995).

Comparing to the control, the application of SWE had a substantial impact on the amount of plant essential oil in terms of biochemical properties as for *Matricarai recutita* (Golzadeh et al., 2012) and *Calendula officinalis* L (Rafiee et al., 2013). Using biofertilizers (50 % *Azotobacter chrococcum* and 50 % *Bacillus megaterium*) has brought about total phenolic compounds and flavonoids higher than that obtained by chemical fertilizers (Naguib et al., 2012).

Table 5: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with three irrigation regimes and eight fertilizer levels

Irrigation regime	Fertilizer Levels	Quantity of leaf extract (g/100 g of air-dried leaves)
W1	F1	4.489±1.48 g*
W1	F2	6.501±0.83 efg
W1	F3	8.248±0.18 abcde
W1	F4	8.320±1.21 abcde
W1	F5	6.786±0.18 defg
W1	F6	8.564±2.00 abcde
W1	F7	8.622 ±0.32 abcde
W1	F8	6.589±2.69 efg
W2	F1	6.758±0.42 defg
W2	F2	7.823±0.49 bcdef
W2	F3	10.050±4.01 abc
W2	F4	10.567±3.43 a
W2	F5	7.422±0.81 cdef
W2	F6	8.251±0.18 abcde
W2	F7	8.321±0.30 abcde
W2	F8	9.272±2.45 abcd
W_3	F1	10.372±1.50 ab
W_3	F2	7.377±0.28 def
W_3	F3	6.544±0.91 efg
W_3	F4	8.483±0.61 abcde
W_3	F5	5.223±0.48 fg
W_3	F6	8.455±1.49 abcde
W_3	F7	7.097±0.87 defg
W_3	F8	5.300±1.31 fg
LSD 0.05		2.640

*: Means with same letter/s are not significantly different according to LSD at 0.05 level of probability.

W_1: 100%Nile water (NW).

W_2: 50% (NW) + 50% Agricultural Drainage Water (ADW)

W_3: 100% Agricultural Drainage Water (ADW)

F_1: Without fertilizer.

F_2: 1g N, 0.5g P_2O_5 and 0.5g K_2O/bag.

F_3: Seaweed extract (SWE) 7ml/ L (Organic fertilization).

F_4: Top Strains of Biotechnology (TS) 30ml/ bag (Bio fertilization).

F_5: Mineral fertilization + Bio fertilization.

F_6: Mineral fertilization + Organic fertilization.

F_7: Bio fertilization + Organic fertilization.

F_8: Mineral fertilization + Bio fertilization + Organic fertilization.

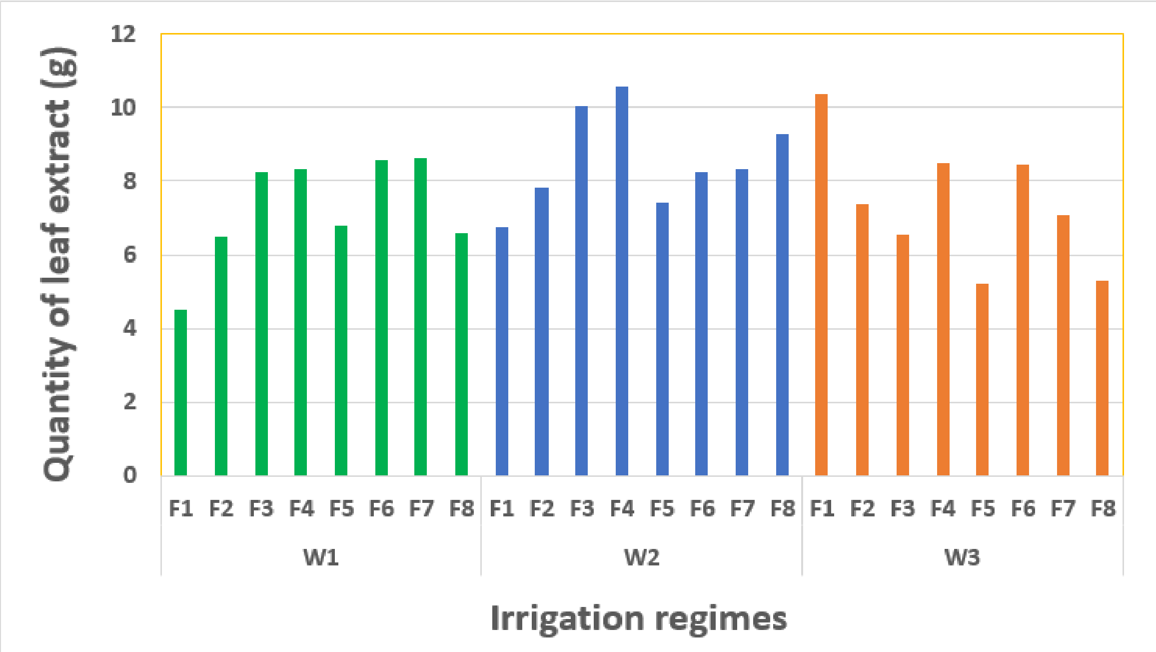

Fig. 4: Quantity of leaf extract (g/100 g of air-dried leaves) of *Khaya senegalensis*, treated with three irrigation regimes and eight fertilizer levels. W1: 100%Nile water (NW). W2: 50% (NW) + 50% Agricultural Drainage Water (ADW). W3: 100% Agricultural Drainage Water (ADW). F1: Without fertilizer. F2: 1g N, 0.5g P_2O_5 and 0.5g K_2O/bag. F3: Seaweed extract (SWE) 7ml/ L (Organic fertilization). F4: Top Strains of Biotechnology (TS) 30ml/bag (Bio fertilization). F5: Mineral fertilization + Bio fertilization. F6: Mineral fertilization + Organic fertilization. F7: Bio fertilization+Organic fertilization. F8:Mineral fertilization +Bio fertilization+ Organic fertilization.

CONCLUSIONS

Our study demonstrated the significant effects of fertilizer types, irrigation schedules, and their combinations on the amounts of leaf extracts obtained from *Khaya senegalensis* seedlings. The seedlings irrigated with 50% Nile Water + 50% agricultural drainage water and fertilized with TS 30 ml/bag showed the highest leaf extract content followed by those irrigated with 100% agricultural drainage water + without fertilizer.

DECLARATIONS

Consent for publication: Not applicable.
Availability of data and materials: All data generated or analyzed during this study are included in this published article.
Competing interests: The authors affirm no conflict of interest.
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AUTHORS' CONTRIBUTIONS

Esraa A. M. Mohamed, carried out the greenhouse experiment; Esraa A. M. Mohamed and Mohamed Z. M. Salem carried out the methodology, Esraa A. M. Mohamed, Ahmed M. El-Baha, Ahmed A. A. El-Settawy, Nader D. Shetta, Mohamed Z. M. Salem investigated the results. Esraa A. M. Mohamed, Ahmed M. El-Baha, Ahmed A. A. El-Settawy, Nader D. Shetta, Mohamed Z. M. Salem, prepared the figures and Tables. All authors shared in writing and reviewing the manuscript.

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الملخص العربي

تقدير كمية المستخلصات الخام من أوراق الكايا سينجالينسيس المروية بمياه الصرف الزراعي والمسمدة بطحالب البحر وبعض الأسمدة الحيوية

اسراء عادل محمود، احمد محمد البحة، احمد على عامر، نادر دسوقى شتا، محمد زيدان سالم

قسم الغابات وتكنولوجيا الاخشاب – كلية الزراعة– جامعة الاسكندرية

في تلك الدراسة، تم استخدام أوراق جافة هوائياً من شتلات الكايا سينجالينسيس التي تم ريها بثلاثة جداول ري مختلف باستخدام مياه الصرف الزراعي، وتم تسميدها بمستخلص الطحالب البحرية، وبعض الأسمدة الحيوية، والأسمدة المعدنية، بالإضافة إلى تركيباتها، لدراسة تأثيرها على كمية المستخلصات الخام. تم إجراء الاستخلاص الخام بإستخدام مذيب الإيثانول وتم التعبير عنه بوحدات جرام لكل ١٠٠ جرام من الأوراق المجففة بالهواء. فيما يتعلق بتأثير نظام الري، فإن الري الذي يستخدم ٥٠٪ من مياه الصرف الزراعي و٥٠٪ من مياه النيل قد أنتج أعلى كمية من مستخلص الأوراق (٨.٥٥ جرام/١٠٠ جرام من الأوراق المجففة بالهواء). أظهرت الشتلات التي تم تسميدها بالسماد الحيوي (٣٠ مل/كيس) الذي يحتوي على سلالات ®(TS) (سائل غني حيوياً، شركة بايو تك) والتي تضم *Bacillus circulans* و*Bacillus polymyxa* و *Bacillus megaterium* و *.Candida spp* و *.Trichoderma spp* أعلى كمية من مستخلص الأوراق، حيث بلغت ٩.١٢٣ جرام/١٠٠ جرام من الأوراق المجففة بالهواء. هذا يدل على التأثير الإيجابي للتسميد. أظهرت الشتلات المروية بمزيج ٥٠٪ من مياه الصرف الزراعي و٥٠٪ من مياه النيل، والمسمدة بـــ ٣٠ مل من السماد ®TS لكل كيس أعلى كمية مستخلص خام (١٠.٥٦ جرام/١٠٠ جرام من الأوراق المجففة بالهواء). يليها تلك الشتلات التي تم ريها بمياه الصرف الزراعي بنسبة ١٠٠٪ بدون سماد (١٠.٣٧ جرام/١٠٠ جرام من الأوراق المجففة بالهواء).

الكلمات المفتاحية: الكايا سينجالينسيس– مستخلص خام– مستخلص إيثانولي– تحليل كمي– محفزات حيوية– الطحالب البنية سارجاسوم– مياه الصرف الزراعي.