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Effect of two green microalgae on the growth and development of *Corchorus olitorius* during the flowering stage.

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Abstract: Excessive chemical fertilizer used in farming to increase crop yields has increased the risk of environmental destruction and has serious negative impacts on human health. To solve these problems, agriculture farming has evolved to using bio-fertilizers, which not only raise productivity, minerals, and organic compounds but also reduce the harmful effects of chemical fertilizers. In this study, two strains of green microalgae had been isolated. Then each algal isolate was grown in BBM media and BFCE, then the pellet of two isolates after growth was taken and resuspended in the irrigation water. The algal treatments enhanced *Corchorus olitorius* growth parameters, as well as an increase in photosynthetic pigments and protein content compared to the control plant during the flowering stage. Based on the obtained results *Chlorella sorkiniana* and *Scenedesmus quadricauda* could be used as biofertilizers**.**

Keywords: Bio-fertilizers, BFCE, *Chlorella sorkiniana, Scenedesmus quadricauda, Corchorus olitorius.*

Introduction

Bio-fertilizers are preparations containing live or latent cells of effective strains of nitrogen-fixing, phosphate solubilizing, or cellulolytic micro-organisms. By fixing atmospheric nitrogen, both in conjunction with plant roots and separately, bio-fertilizers play a very important part in increasing soil fertility. They also solubilize phosphates insoluble in the soil and form chemicals that help plants flourish. They are in fact encouraged to utilize the biological system of nutrition mobilization that is naturally occurring and readily available [1]. Actually, organic and chemical fertilizers are very different from biofertilizers; organic fertilizers come from either plant or animal sources, such as green manure, or from animal sources such as animal dung [2]. Chemical fertilizers, however, are the major ingredients of non-organic fertilizers salts of phosphate, nitrate, ammonium, and potassium [3]. Chemical fertilizer use has dramatically increased over the past three decades, raising serious concerns. Fertilizers containing nitrogen (N) and phosphorus (P) are now used more frequently than ever before. In addition to air pollution, reduced biodiversity, and suppressed ecosystem function, the increased use of fertilizers and highly productive systems has also led to environmental issues such as soil quality, surface water quality, and groundwater quality degradation. On the other hand, excessive and long-term use of chemical or synthetic fertilizers leads to increased environmental pollution, which will eventually lead to an imbalance in the ecosystem [4]. Sustainable agriculture methods are obviously urgently needed on a worldwide scale. It has long been known that biopesticides and biofertilizers have significant potential for advancing sustainable agriculture [5]. For the plant to develop and flourish properly, 17 vital ingredients are needed. Among these, relatively high amounts of nitrogen (N), phosphorus (P), and potassium (K) are required [6]. Numerous microorganisms, such as cyanobacteria, which dissolve phosphate and are utilized in conjunction with molds and fungi, are frequently used as biofertilizers [7]. Similar to this, bacteria that produce phytohormones are also used in the creation of biofertilizers. They give the plant nutrients that encourage growth, such as indole acetic acid (IAA), amino acids, and vitamins, and they enhance the soil's fertility and production while still maintaining the crop [8].

Corchorus olitorius is a significant green leafy vegetable. As a leafy vegetable, it is also grown in the Caribbean, Brazil, India, Bangladesh, China, and the Middle East. The leaves, which are rich in vitamins and minerals and can be used fresh or dried, are boiled into a thick, viscous broth or added to stew or soup [9].

In addition to being used for cooking, *Corchorus olitorius* is also used as an herbal cure for pains, chronic cystitis, and fevers. In some regions of Nigeria, leaf decoctions are also used to treat ascites, dysuria, pectoral discomfort, and female sterility in addition to treating iron and folic acid deficiencies [10]. The leaves are also used as an herbal remedy to cure typhoid and malarial fevers [11]. While leaf infusion is used to increase hunger, leaf twigs are used to cure heart problems. The leaves are used to treat constipation in Tanzania. Additionally, leaves are employed in Benin to treat infantile malnutrition and as an emollient and diuretic [12]. So, the aim of this study was designed to assess the efficacy of *Scenedesmus quadricauda* and *Chlorella sorkiniana*, as a biofertilizer to *Corchorus olitorius.*

Materials and methods

A semi-field experiment was performed in the greenhouse of the Botany Department, Faculty of Science Mansoura University to test the effect of the test microalgae; *Chlorella sorkiniana،* and *Scenedesmus quadricauda* on the growth and metabolism of the *Corchorus olitorius* plant.

The experiments were done with a lot of homogeneous *Corchorus olitorius* seeds. This pure strain of uniform seeds was taken from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Freshwater samples were collected from the Delta Company for Fertilizers and Chemical Industries. The beet filter cake extract was obtained from Daqahlia Sugar Company.

Time Course of Experiment:

A Group of *Corchorus olitorius* seeds with homogenous size was selected then surface sterilized by soaking the seeds in 0.01 % $HgCl_2$ solution for 3 minutes. The seeds were washed thoroughly with tap water then divided into five equal groups. All sets of seeds were sown in similar earthenware pots filled with equal amounts of garden soil (prepared by mixing clay to sand, 2:1; w/w).

The experiment design was as the following:

T1: Control (plant irrigated with tap water)

T2: Plant irrigated with *Scenedesmus quadricauda* that was grown in BBM media.

T3: Plant irrigated with *Scenedesmus quadricauda* that was growing in 75% concentration of beet filter cake extract (BFCE).

T4: Plant irrigated with *Chlorella sorkiniana،* that grew in BBM media.

T5: Plant irrigated with *Chlorella sorkiniana،* that grew in 50% concentration of beet filter cake extract (BFCE).

All groups of *Corchorus olitorius* seeds were cultivated on the first of August ٢٠٢٢. Irrigation by different microalgae was performed four times; the first irrigation was on the 30th of August, the second irrigation was on the 6th of September, the third was on the 13th of September and the last was on the 20th of September.

Preparation of the microalgae:

A week before the experiment, the algae were prepared for irrigation as follows:

1- 10 liters of *C. sorkiniana،* was grown on BMM media

2- 10 liters of *C. sorkiniana* was grown on a concentration of 50% of BFCE

3- 10 liters of *S. quadricauda* was grown on BBM media

4- 10 liters of *S. quadricauda* was grown on a concentration of 75% of the BFCE

The OD of all Treatments was fixed at approximately 1.

On the morning irrigation day, a centrifugal process was done for the treatments and a pellet was taken and re-dissolved in 10 liters of water that was used for irrigation.

Sample collection:

On the ٣٠th of September 2022 (٦٠ days old) sampling from treated and untreated plants was undertaken, which represent flowering stages. The collected samples were used for the

determination of growth parameters (shoot length, number of leaves/ plant, leave area/plant, shoot fresh weight, shoot dry weight, shoot water percentage, root length, root fresh weight, root dry weight, root water percentage, root length/shoot length) as well as some metabolic aspects for the shoot only such as the changes in pigments content (chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/ chlorophyll b, carotenoids& total pigments), and total proteins.

Biochemical analysis of plant tissue

1. Photosynthetic pigments analysis

The pigments were extracted and determined according to the method of [13].

2-Determination of crude protein

Crude protein was determined by the method of [14] and modified by [15].

Result and Disscusion:

Changes in growth parameters

The changes in the estimated growth parameters (shoot length, number of leaves, leave area, shoot fresh weight, shoot dry weight, shoot water percentage, root length, root fresh weight, root dry weight, root length/ shoot length, and root water percentage) of Corchorus olitorius plant in response to different treatments, during the flowering stages, are displayed in

Table *1* and

Table *2*

Shoot length

At the flowering stage, the shoot length of the plant appeared to increase significantly under the effect of all the used treatments compared to the control (T1). T3 gave the highest shoot length, followed by T2, then T4, and T5.

Number of leaves/plants

As compared to the control value, the number of leaves increases significantly with all treatments in the flowering stages. T3 gives the highest number of leaves, and the control gives the lowest number of leaves.

Leave area/plant

All algal treatments significantly increase the leaf area during the flowering stages compared to the control. T3 gave the highest leaf area, followed by T5 then T2, and T4.

Shoot fresh weight

Compared to the control value and during the flowering stage of *Corchorus olitorius* growth and development, a significant increase in total shoot fresh weight/plant was observed in all treated plants with T3, T2, T4, and T5, respectively. The plant treated with T2, and T3 recorded a fresh weight of the shoot nearly triple the value of the control, while the plant treated with T4, and T5 recorded double the value of the shoot fresh weight of the control.

Shoot dry weight

Plants treated with the four algal treatments record a root dry weight significantly higher than the control. The sequence of increments was T3, T2, T4, and T5 then control.

Shoot water percentages

At the flowering stage, all the treatments non-significantly decreased shoot water percentages, except T5 decreased the percentage significantly compared to the control. The maximum percentage was the control at the flowering stage.

Table 1 Effect of microalgal treatments on growth parameters of *Corchorus olitorius* shoot during the flowering stage.

Treatment	Fertilizer	Shootlength (cm/plant)	Number of leaves/plants	Leavesarea (cm/plant)	Shootfresh wt.(g/plant)	Shoot dry wt.(g/plant)	Shootwaterpercentage%
T1	Negative	28.9^{c}	27^{b}	15.36^{b}	3.3^{d}	0.41^{d}	$87.0\%^{a}$
T2	*S.quadricauda*	49.9^{a}	58^{a}	23.83^{a}	9.62^{b}	1.26^{ab}	$86.8\%^{a}$
T3	*S.quadricauda* treated with 75% BFCE	50.6^{a}	60^{a}	26.6^{a}	10.83^{a}	1.47^{a}	$86.4\%^{a}$
T4	*C.sorokiniana*	41.6^{b}	55^{a}	22.87^{a}	7.89^{c}	1.05^{ab}	$86.6\%^{a}$
T5	*C.sorokiniana* treated with 50% BFCE	38.4^{b}	52^{a}	24.87^{a}	7.18^{c}	0.99^{c}	$85.9\%^{b}$

Root length

All used treatments increased the root length significantly throughout the flowering stage. T3 and T4 lead to a doubling of the root length compared to the control.

Root fresh weight

T2, T3, T4, and T5 gave a significant increase compared to T1 in the root fresh weight. T3 gave the highest root fresh weight, followed by T2, T4, and T5, respectively, with convergent values.

Root dry weight

Plants treated with algae give a significant increase in root dry weight than the control plant, and the highest root dry weight among the algal treatments was T3.

Root length/shoot length

At the flowering stage T2, T3, and T5 non-significantly increase, T4 recorded a significant increase in root length/ shoot length than T1.

Root water percentage%

At the flowering stage, T2, T3, T4, and T5 caused a significant decrease in root water percentage than the control. The highest root water percentage was T3 and T1 respectively.

Table 2 Effect of microalgal treatments on *Corchorus olitorius* root growth parameters during the flowering stages.

Treatment	Fertilizer	Root length (cm/plant)	Root fresh wt.(g/plan)	Root dry wt. (g/plant)	Root length/ Shoot length	Root water percentage%
T1	Negative	10.2^{c}	0.42^{d}	0.053^{c}	0.356^{b}	$86.9\%^{a}$
T2	*S.quadricauda*	17.4^{ab}	0.78^{b}	0.15^{b}	0.359^{b}	$79.8\%^{b}$
T3	*S.quadricauda* treated with 75% BFCE	19.6^{a}	0.94^{a}	0.20^{a}	0.391^{ab}	$78.5\%^{b}$
T4	*C.sorokiniana*	19.5^{a}	0.64^{c}	0.12^{b}	0.472^{a}	$79.7\%^{b}$
T5	*C.sorokiniana* treated with 50% BFCE	15.8^{b}	0.66^{c}	0.14^{b}	0.416^{ab}	$78.8\%^{b}$

Changes in photosynthetic pigments contents

The effect of microalgae on photosynthetic pigments (Chlorophyll a, chlorophyll b, total chlorophylls, carotenoids, and total pigments) which were determined in leaf samples of cultivated *Corchorus olitorius* during flowering stages were shown in

Protein content

T2 at the flowering stages caused a significant increase in protein content compared to the control (T1). T4 and T5 caused a non-significant decrease the protein content The highest protein value was recorded with T2. It could be concluded from this study that *Chlorella sorkiniana* and *Scenedesmus quadricauda* could be used as biofertilizers as they improve growth and metabolism of *Corchorus olitoriu*

Table *3*

Chlorophyll a

The used treatments led to a significant increase in the chl. A content, except T3, caused a non-significant increase. The highest chl.a value recorded by T4.

Chlorophyll b

The used treatments caused a non-significant increase in Chl. b content compared to the control plant (T1) except T4 caused a significant increase and T2 cause a significant decrease in chl.b value compared to T1.

Chlorophyll a/ Chlorophyll b ratio

In comparison to control values of chlorophyll a/ chlorophyll b ratio was affected by the used treatments at both stages. This ratio increased significantly and non-significantly in response to treatment with T2 &T5 respectively. but decreased non-significantly under treatments with T3, &T4.

Total chlorophylls

At the flowering stages a significant increase in total chlorophylls in response to all algal treatments. T4 gave the highest percentage of total chlorophylls.

Carotenoids

The used algal treatments cause a non-significant increase in the content of carotenoids except T4 caused a significant increase compared to the value of T1. T4 recorded the highest carotenoid value.

Total pigments

The total pigment content of *Corchorus olitorius* leaves was consequently significantly increased during flowering stage by all treatments compared to the control value (T1), except T3 caused a non-significant increase.

Protein content

T2 at the flowering stages caused a significant increase in protein content compared to the control (T1). T4 and T5 caused a non-significant decrease the protein content The highest protein value was recorded with T2. It could be concluded from this study that *Chlorella sorkiniana* and *Scenedesmus quadricauda* could be used as biofertilizers as they improve growth and metabolism of *Corchorus olitoriu*

Table 3 Effect of microalgal treatments in photosynthetic pigments of *Corchorus olitorius* leaves during flowering stages (expressed as mg/g fresh wt.)

Treatment	Fertilizer	Chl. a	Chl. b	Chl. a/Chl.b	Chla+Chl.b	Carotenoids	Totalpigments
T1	Negative	15.9^{b}	9.6^{b}	1.7^{bc}	25.5^{e}	9.2^{b}	34.7^{d}
T2	*S.quadricauda*	19.1^{a}	8.5^{c}	2.2^{a}	27.6^{c}	9.9^{b}	37.5^{bc}
T3	*S.quadricauda* treated with 75% BFCE	16.3^{b}	10.3^{b}	1.6^{c}	26.6^{d}	9.4^{b}	36.0^{cd}
T4	*C.sorokiniana*	19.2^{a}	12.6^{a}	1.5^{c}	31.8^{a}	11.8^{a}	43.6^{a}
T5	*C.sorokiniana* treated with 50% BFCE	18.8^{a}	10.0^{b}	1.9^{b}	28.8^{b}	9.9^{b}	38.7^{b}

Table 4 Effect of microalgal treatments in total protein content of *Corchorus olitorius* shoot during flowering stages (expressed as mg/g fresh wt.)

Treatment	Fertilizer	Total protein at flowering stage (mg/g fresh wt.)
T1	Control	20.27^{b}
T2	*S. quadricauda*	22.11^{a}
T3	*S.quadricauda* treated with 75% BFCE	21.05^{b}
T4	*C.sorokiniana*	19.14^{c}
T5	*C.sorokiniana* treated with 50% BFCE	18.95^{c}

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