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Stimulatory effects of alga *Spirogyra* extract on *Glycine max* L.

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ABSTRACT

This study aimed to determine the effects of *Spirogyra* extract on plant growth, yields and nutritional and nutraceutical traits of soybean seeds. An experiment was done during 2022-2023 Soybean seeds of Taqa and Lee 74 cultivars were sown, and the *Spirogyra* extract (0.5, 1.5, 3 and 6 %) was used to irrigate to 75 % per pot) Field capacity. Biometric traits, seed yield, and seed number were determined. The application of *Spirogyra* extract decreased the growth and yield of soybean, with no adverse effects on the nutritional value of seeds. Allelopathic compounds present in the algae extract were identified using HPLC, and included hydroquinone and cinnamic acid.

Key words: Alga, allelopathic compounds, Chlorophyll, HPLC, *Glycine max*, nutraceutical traits, plant growth, *Spirogyra*, Stimulatory effects, yield.

INTRODUCTION

Soybean (*Glycine max* L.) Fabaceae family (Figure 1a), is the fourth crop in the world after rice, wheat and maize. It is an essential source of plant-based protein for human diet and also used to make to wide range of chemicals (14). Its seeds are rich in vital amino acids, vitamins, and unsaturated fatty acids such as oleic and linoleic acids, with significant percentages of oil (14-24 %) and proteins (30-50 %) (13). Soybean is a multipurpose crop for food, industrial, and fodder uses and used in the human food industry and as a source of protein in animal diets. The growing need for protein in fodder

a **b**

Figure 1. Test plants used in experiment: a (Soybean), b (*Spirogyra*)

led to an expansion in soybean farming (7). Farmers are using more nitrogen fertilizers. Considerable energy is required to produce nitrogen fertilizers from fossil fuels like coal and oil makes them expensive. The careless use of these fertilizers have impacted the ecosystems of soil and water.

During their growth, algae, e.g. genus *Spirogyra* (Figure 1b), produce a variety of secondary metabolites (fatty acids, polysaccharides, phenolic compounds, and carotenoids). Their biosynthetic levels depends on the surrounding conditions. These secondary metabolites, together with bioremediation and fertilizing qualities, also offer antioxidant, anticancer, and antibacterial activity. This study aimed to determine how *Spirogyra* extracts affected the germination and development of soybeans.

MATERIAL AND METHODS

This study was done in greenhouse conditions at Department of Biology, Faculty of Science, Mosul University, Iraq on 25/2/2021. *Spirogyra* (*Zygnium* family, Zygnium order) was collected from the pond surface and used to produce the algal extracts. The algal biomass was dried and powdered to prepare the aqueous extracts.

Preparation of *Spirogyra* aqueous extracts

Distilled water was added to the sieved algal biomass to obtain concentrations of 0.5, 1.5, 3, 6 g/100 ml. Then, the aqueous extracts were filtered using Whatman No. 1 filter paper. The clear liquids collected for each concentration were kept in the refrigerator at 4 °C until use (9).

Petri-plate Laboratory experiments

Petri dishes were used and 15 seeds were placed in each dish between two layers of filter paper. Eight ml aqueous extracts (0.5, 1.5, 3 and 6 g/100 ml) were added per dish. For every aqueous extract tested, 3-dishes were used. After that, dishes were kept at 25 ± 2 ºC in a Gallenkhamp incubator. After 7-day incubation period, germination (%) was assessed, the seedlings were thinned to 5 individuals per dish. The lengths of the stems and roots were also measured (15 days after sowing). After drying for 72 h at 60 °C in oven, the seedlings' dry weight was recorded (10). The germination (%) was calculated as under :

Germination (%) = Number of seedlings / Number of seeds sown x 100 (16)

Pot culture Greenhouse experiments

Soybean seeds were sown on 9/3/2022 in plastic pots of (20 cm dia and 25 cm height) filled with 5 kg soil and 10 seeds were sown per pot. The aqueous extracts of *Spirogyra* (0.5, 1.5, 3 and 6 g/100 ml) were used to irrigate Field capacity to 75 % per pot. Three pots were prepared for each extract tested. Pots were kept in greenhouse temperature 25-30 °C, and irrigated till the duration of the assay 12/7/2022 The Germination (%) was calculated 8 days after sowing. Plant samples were collected after 90 days. Shoot height (cm), root length, plant dry weight (g) and leaf area (cm^2) were calculated as per Saieed method (17). Yield components (No. of seeds, seed weight / pod (mg), pod length, No. of pod, pod weight (mg), Total chlorophyll in fresh leaves were determined according to (12).

Some physical and chemical traits of experimental Clay silt soil, treated with *Spirogyra* extract at (0.5, 1.5, 3, and 6 %) were determined in Department of Soil Sciences, College of Agriculture and Forestry, University of Mosul. The NPK was determined in the leaves of soybean plants treated with *Spirogyra* extract at 0.5, 1.5, 3, and 6 % in both test cultivars according to (N): Kjeldall method (11), (P) by Olsen (15), (K) by Hussain (9) methods.

Identified Phenolic compounds

Algal ethanolic 70 % extract was prepared as per the method of Al-Juhaishi (6). Standard solutions of hydroquinone, *p*-hydroxybenzoic acid, vanillin, salicylic acid, benzoic acid, cinammic acid, gallic acid, ferulic acid, chlorogenic acid and quercetin were prepared at concentrations of 0.01 g/100 ml of distilled water. HPLC analysis was done in College of Agriculture and Forestry, University of Mosul. The apparatus was a Shimadzu corporation A 2010 C18 column (6 mm in dia, 5 µm particles; Macherey-Nagel) 35 °C, mobile phase consisted in 90 % acetonitrile and 10 % water, at a flow rate of 2 ml/min, with a cell volume of 2 ml. Samples and standards were injected at a volume of 5 microliters and separation was monitored at 320 nm.

Statistical analysis : All data were statistically analyzed by using SAS software (SAS). Duncan's Multiple Range Test was applied to compare the means at 0.05 probability level.

RESULTS AND DISCUSSION

Table 1 outlines the impact of *Spirogyra* extracts on soybean germination and seedling growth. In cultivars Taqa and Lee74 the highest percentages of germination, elongation and dry weight were recorded at 0.5 % concentration (100 %, 11.25 % and 63 %, respectively). Perhaps because, allelochemicals in the extracts have hormetic effects at low concentrations, which may have caused the bio-stimulation (1). This beneficial effect is further enhanced by the vitamins, minerals, and growth hormones (cytokinins, polysaccharides, and amino acids) that algae contain and which are vital for plant growth (8). On the other hand, *Spirogyra* extracts at higher concentrations of 6 % Caused total germination inhibition and reduced all growth parameters. These promising results led us to perform the greenhouse experiments (Table 2), where the 0.5 % aqueous extracts increased all phenotypic traits (Germination percentage., SL, RL, and No. of Leaves) in both soybean cultivars. Taqa cultivar surpassed Lee74 in all examined traits (Germination (%), Roots Length, Roots Dry Wight, Plumule Length, Plumule Dry Wight) by 54.36 %, 52.38 %, 16.52 %, and 39.13 %, respectively, presumably due to genetic differences (6). At 1.5 %, 3 % and 6 % concentrations, these features declined with increase in concentration, so did the degree of inhibition. These results are consistent with finding (5), which showed that *Brassica napus* seedling height and shoot dry weight were considerably increased by foliar spraying algal extract than control. This enhancement could be attributed to the major and minor nutrients present in the extract, which influences photosynthesis, cellular metabolism, production of proteins, nucleic acids, enzymes, and hormones, ultimately improving the growth of the root and shoots (2,18). The applied *Spirogyra* aqueous extract increased the yield components (No. of seeds, Seed Weight/pod (mg), Pod length, No. of the pod, pod Weight (mg)] (Table 3). At the 0.5 % concentration, the highest increase was in seed weight/pod (60 %) and pod length (61 %) in Taqa

Table 1. Effects of *Spirogyra* extract in germination of *Soybean*

Extract Conc (%)	Germination (%)	Roots		Plumule	
		Length (cm)	DW (mg)	Length (cm)	DW (mg)
	Taqa variety				
Control	80b	5b	0. 06	6.8b	50c
0.5	89a	8.15a	4a	9.0a	80a
1.5	52c	2.9c	1b	4.0c	53b
3.0	36d	2.2c	0.02c	3.4d	36d
6.0	11.5e	0d	0d	0e	0e
	Lee 74 variety				
Control	77b	3.3 b	0.04 b	2.8 b	63a
0.5	88a	4.3 a	7 a	5.9 a	28c
1.5	58c	2.5 c	0.03cb	2.1cb	43b
3.0	46 d	2.0 d	0.02 c	2.1 cb	23d
6.0	5 e	0 e	0 e	0 e	0e

DW: Dry weight

Table 2. Effects of *Spirogyra* extract in morphology traits of *Soybean*

Extract Conc (%)	Germination (%)	Roots Length (cm)	Shoot Length (cm)	No. of leaf
	Taqa variety			
Control	51.5 b	6.3 b	60.5 b	23 b
0.5	79.5a	9.6 a	70.5 a	32.0 a
1.5	43 c	7.8 c	51.5 c	17 c
3.0	18 d	6.2 d	41 d	16 d
6.0	13e	3.5 e	27 e	14 e
	Lee 74 variety			
Control	51.5b	6.2 b	42 b	23 b
0.5	76.5 a	9.1 a	46.5 a	29 a
1.5	23d	8.1 c	33.5 c	24 c
3.0	26.5 c	6.2 d	32.5 d	18 d
6.0	19 e	4.4 e	27.5 e	16 e

3. Effects of Spirogyra extract in yield components of *Soybean* Table

Extract Conc (%)	No. of seeds Seed/ pod	Seed Weight / pod (mg)	Pod length	No of pod	Pod Weight (mg)
	Taqa variety				
Control	1 b	7 b	2.5 b	5 b	24 b
0.5	2a	18 a	4.0 a	7 a	56 a
1.5	0 c	0 c	0 c	0 c	0 c
3.0	0 c	0 c	0 c	0 c	0 c
6.0	0 c	0 c	0 c	0 c	0 c
	Lee 74 variety				
Control	1b	6 b	2.5 b	3 b	8 b
0.5	2a	9 a	3.6 a	6 a	18 a
1.5	0c	0 c	0 c	0 c	0 c
3.0	0c	0 c	0 c	0 c	0 c
6.0	0c	0	0	0	0

variety. Yield components were reduced at extracts of higher concentration, which could be due to both an increase in allelochemicals concentrations and soil nitrogen content. The highest rate of increase in pods weight was 10 % for Taqa cultivar at the concentration of 0.5 %. In fact, this concentration was the only one that increased yields of all traits in both soybean cultivars likely due to a promotive effect of algae constituents on many physiological plant processes (14).

Table 4 highlights the effect of *Spirogyra* extract on leaf area and photosynthesis pigments of Soybean. When algal extracts were foliar sprayed at concentrations more than 0.5 %, photosynthetic pigments (carotenoids, Chl *a*, and Chl *b*) and leaf area decreased. Allelopathic chemicals, such as hydroquinone, which was detected in the current study by HPLC Figure 2 and is known to have an impact on stomata, gas exchange and guard cell distribution, hence hindering growth and reducing leaf area (8), might be to responsible for this decline. On the other hand, a 0.5 % concentration increased the amount of photosynthetic pigments and leaf area, presumably because allelopathic chemicals affect the mechanisms that produce enzymes and chlorophyll (19).

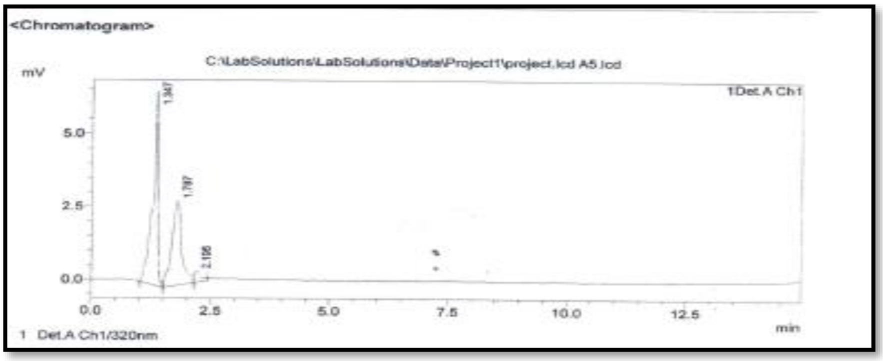
Table 4. Effects of Spirogyra extract in leaf area and photosynthesis pigments of *Soybean*

Extract Conc (%)	**Leaf area (cm^2)**	**Chl. A**	**Chl. B**	**Totle Chl.**	**Carotenoids**
	Taqa variety				
Control	1.15	20.3	52.4	72.7	3.9
0.5	1.48a	22.5 a	54.1 a	76.5 a	4.6 a
1.5	0.92 c	20.5b	50.4 c	71.5b	4.1b
3.0	087 c	20.6b	50.6 c	71.2b	3.5 c
6.0	0.75d	16.4d	40.1 c	56.5 c	3.5
	Lee 74 variety				
Control	1.0b	17.3 c	52.1 b	72.5 b	3.8
0.5	1.27 a	21.8 a	54.7 a	74.5 a	4.5 a
1.5	0.75c	20.7 b	51.2 b	72.1 b	4.0 b
3.0	0.65 c	19.2 c	50.5d	69.7d	4.0
6.0	0.6 c	19.0 c	53 c	73 c	3.6

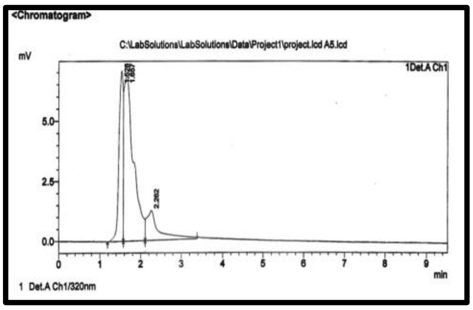
Spraying soybean with *Spirogyra* extract caused an increase in soil N content (Table 5), which increased with increasing concentration of algae biomass. P content decreased at concentrations of 0.5 % and 1.5 %, but increased at concentrations of 3 % and 6 %, suggesting that the algae extracts, rich in various nutrients, were not only absorbed by the leaves but also improved soil fertility status (9). The K content decreased at all concentrations, and the highest rate of decrease was 66 % at 6 % extract concentration. This effect may be due to the presence of the cinnamic acid identified by HPLC (Fig. 2). Cinnamic acid is also able to influence the net uptake of nitrate and ATPase activity in the target plants (3).

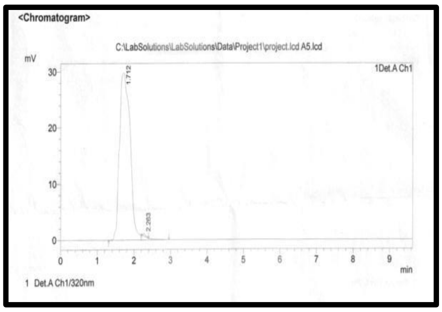
Table 5. Effects of Spirogyra extract in percentage of elements in soil *Soybean*

Treatments	N (%)	P (%)	K (%)
Control	0.015 d	0.0015 b	0.0054 a
0.5	0.015d	0.0006 c	0.005e
1.5	0.023c	0.001 d	0.0027 b
3	0.031b	0.002 d	0.0019 c
6	0.039 a	0.0022 a	0.0018 d



(a)





(b) (c)

Figure 2. Absorbance peaks of HPLC-separated standard samples of Spirogyra extract. and for each of (a) standard sample (b) Hydroquinone and (c) cinnamic acid

Table 6 showed the effects of spraying with *Spirogyra* extract on the leaf content of NPK in the two soybean cultivars. At 0.5 %, Taqa and Lee74 increased the P content in the plants, while at 1.5 %, Taqa exhibited a high percentage of NK, while Lee 74 had a high percentage of NK at 0.5 %. A high percentage of N was recorded at 6 % concentration. Physiological and genetic variations may explain the observed differences. Concentrations other than 0.5 % showed a reduced the nutrients concentrations, possibly due to allelopathic compounds affecting NPK absorption from soil.

Table 6. Effects of Spirogyra extract on elements (%) in *Soybean* leaf

Extract Conc (%)	**Protein (%)**	**K (%)**	**P (%)**	**N (%)**
	Taqa variety			
Control	17.75 c	0.82 c	0.61d	2.84c
0.5	18.37 b	0.86 c	0.81 a	2.94b
1.5	23.43a	0.94 a	0.71 c	3.75a
3.0	17.12 c	0.92 b	0.81a	2.74d
6.0	12.25d	0.79 d	0.6 d	1.96 e
	Lee 74 variety			
Control	14.68	0.84	0.75	2.35 b
0.5	12.81 c	1.28 a	1.11 a	2.05d
1.5	17.75a	1.00 b	0.90 b	2.84b
3.0	15.31 b	0.81d	0.80 c	2.45c
6.0	12.43d	0.92 c	0.81 c	3.43 a

CONCLUSIONS

Our results suggested that *Spirogyra* biomass has the potential as biofertilizer, when applied at a specific concentration, increasing yield and quality of soybean seeds. Nutritional status and total proteins were increased in soybean irrigated with Spirogyra extract at 0.5 %. Hydroquinone and cinnamic acid were also identified in *Spirogyra* extract by HPLC.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

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