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Evaluation of The Spirulina (*Arthrospira platensis* Gomont) Antimicrobial Activity

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ABSTRACT

The present work was carried out to enhance biomass production of Spirulina (*Arthrospira platensis* Gomont) in a modified medium and to investigate "*in vitro"* its ability to produce antimicrobial substances against pathogenic bacteria and phytopathogenic fungi. The antibacterial activity was evaluated by the solid medium diffusion method on two pathogenic bacteria (*Bacillus cereus & Klebsiella sp.*), And the antifungal activity was evaluated on three phytopathogenic fungi (*Alternaria solani, Cladosporium sp., Fusarium culmorum).* The antibacterial test showed that the aqueous extract produced by *S. platensis* was more active against Gram-positive than on Gram-negative bacteria, the highest antibacterial activity was recorded against *Bacillus cereus*. Moreover, the Antifungal test showed that the aqueous extract was active against all three tested fungi and the highest antifungal activity was recorded against *F. culmorum* with over 90% inhibition of mycelial growth. The results of this research proved that cyanobacteria could be a good source for the production of antimicrobial agents which could be effective when compared with contemporary antimicrobial compounds and it can be used in the Biocontrol of several plant fungal diseases.

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Introduction

The emphasis in the last decade has been focused on the natural antimicrobial components produced by aquatic organisms. Cyanobacteria are the most potent sources of high-value chemicals, pharmaceuticals, and antimicrobials (Bancalari et al. 2020). Approximately 25.000 algae are known in the last 3 decades, among them, we can distinguish a microscopic blue-green alga that appeared to be the first living beings about 3.5 billion years ago and was considered to be the most natural food (Le Bras et al., 2016). It is the Cyanobacterium *Arthrospira Platensis*, better known under the name of *Arthrospira platensis* Gomont. This latter is regarded as a food resource with an abundance of up to 70% protein; rich in mineral salts; trace elements and numerous vitamins Like B1, B2, B12, and E (Matufi et al., 2020).

According to UNESCO (United Nations Educational, Scientific and Cultural Organization), it is "the ideal and most complete food of tomorrow" and for the WHO (World Health Organization)"this is the best food for mankind in the 21st century " (Manet, 2016).

Spirulina has arisen as one of the best synthesizers possible for new therapeutic compounds. It is recognized to produce intracellular and extracellular metabolites with diverse biological activities such as antifungal and antibacterial activities (Al-ghanayem, 2017).

It was also used as an adjunct dietary ingredient of feed for fish, shrimp and poultry, and increasingly as a protein and vitamin complement to aqua feeds. China makes partial use of this micro algae extra of imported feed to promote viability; immunity and the growth of shrimp (Girardin-Andréani, 2005).

In Algeria, there is a growing interest for the Spirulina and many researchers are developing new methods to produce these algae, which is already exists (Boukhari et al., 2018, Aouir, 2017, Saggaï et al., 2016).

This work aims to improve the culture of Spirulina, to investigate the antifungal and antibacterial activity of three different aqueous extract of the cultivated cyanobacterium.

Materials and Methods

Microbes Material

The strain of Spirulina used in our work was Spirulina powder samples packaged in 200g bag (Naturya®).

All the fungal strains were isolated and identified from durum wheat seeds in the laboratory of applied microbiology. The used fungi were: *Fusarium culmorum*, *Alternaria solani* and *Cladosporium sp*.

The bacterial strains were obtained from the laboratory of applied microbiology. *Bacillus cereus* B_{14} and *Klebsiella sp.* Were used as Gram-positive and Gram-negative bacterial strains successively.

Culture Media

The culture medium retained was that of SEGGAI (2008), with slight modification. This medium is the most suitable for the culture of *S. plantesis* in the region of Ouargla province in Algeria, where the composition (g/L) was: $(NH_4)_2SO_4$ 0.2, $FeSO_4$ 0.05, K_2SO_2 0.1, $NaHCO_3$ 04, Na_2CO_3 $10H_2O$ 08. The Potato Dextrose Agar (PDA) and Mueller Hinton Agar (MHA) media were used for the antifungal and antibacterial activity test successively.

Preparation of spirulina Solution in Bicarbonate Water

Under the conditions of sterility, 10 g of Spirulina's powder was divided and placed in three 500 mL glass flasks, containing each one, 250 mL of distillated water, and then 25 g of pre-sterilized bicarbonate was added, then mixed until a homogeneous liquid was obtained; after incubation at 37°C for 48 h, centrifugation was carried out, the supernatant was removed, then the pellet was collected and washed twice with sterile physiological water.

The pellet thus obtained was placed in a 250 mL glass flask of pre-sterilized 10% bicarbonate solution, then incubated at 37°C for 72 hours; the mixture was then centrifuged (3000 rmp for 20 min), and the supernatant was removed, and finally the pellet was washed twice with physiologically sterile water. the recovered pellet was, then, resuspended in 100 mL of physiological water and incubated at 37°C before starting the culture (used as inoculum).

Procedure of Culture

The culture of Spirulina was started with a volume of 120 mL of Spirulina strain (the inoculum), to which a volume of 600 mL of SAGGI culture medium was added (1 volume of strain for 5 volume of culture medium).

The experimental set-up was installed on a table in a heated small room. The strain was grown in a 2-liter plastic box and put inside another 10-liter plastic box that contained distilled water. A constant temperature of 37 °C and agitation was provided by an automatic agitator to ensure good ventilation, the light was provided using a LED lamp of 15W (1200 Lux), placed up to 15 centimeters to the culture.

Preparation of The Aqueous Extract

The investigation of the antibacterial and antifungal activity was based on 3 types of extract:

- *Type 01:* Inoculum gained after reactivation of S. platensis in bicarbonate for 7 days (a theory to compare the effect of the biological activity in 7 and 30 days, whether S. platensis produce different component directly after its reactivation or after a while)
- *Type 02:* Spirulina culture for 30 days infiltrated (cells+ metabolites)
- *Type 03:* Spirulina culture for 30 days was centrifugated (3000rmp/20 min). The supernatant of the culture was filtrated by 0.22 µm filter (Only the metabolites).

Antimicrobial Test

Antibacterial activity

Antibacterial activity of the three Spirulina aqueous extracts, against the two bacterial strains was carried out using the same method as the antifungal activity test. Fresh bacterial culture (24h) was streaked on the culture media containing 12 mL of molten MHA and 3 mL of Spirulina extract. After 24h of incubation at 30C°, the antibacterial effect was observed in comparison with control where bacteria were streaked on MHA media. Each test was performed in triplicate.

Antifungal activity

Antifungal activity of the Spirulina aqueous extract, against the three strains of fungi (cited above), was made by following the method proposed by Li XF et al., (2008). The test was adjusted by standardization method on solid medium.

From each Spirulina aqueous extract, a volume of 3 mL was mixed with 12 mL of molten PSA (Potato's Sucrose Agar). After the petri dishes are dry, a disc of 6 mm diameter from each fungi 7-days-culture was put in the middle of the petri dish. (Three dishes were used for each fungus).

the antifungal activity was evaluated after 7 days and 14 days of incubation in 25C° and 12/12 of light and obscurity. The results were expressed as the inhibition percentage of the mycelium radial growth in comparison with controls, where a disc from fungi culture was put in the middle of the petri dish containing PSA media only.

Statistical Analysis

Test of ANOVA multiway, was used to evaluate the difference between the variants using SPSS version 25 Software.

Results and Discussion

Reactivation of the Spirulina

Spirulina was able to grow in modified media. This was indicated by the increase of optical density (OD) during cultivation. Periodic observation with an optical microscope allowed us to monitor the morphology of Spirulina throughout the culture period, shows the morphological change of Spirulina. A significant increase

in biomass production was observed, the culture medium gave a much higher yield of Spirulina than the started powder weight (10 g), this latter reached 22.6 g of pure weight after centrifugation. The Spirulina growth was more than double (226 %).

Antibacterial activity of Spirulina

The antibacterial activity of Spirulina was assayed against two pathogenic bacterial strains (*B. cereus* and *Klebsiella sp.)* by the evaluation of the inhibition of the bacterial growth. The results showed that Spirulina exerts a biological inhibitory activity against both bacteria strains. The inhibitory effect was mostly against *B. cereus* compared to the *Klebsiella sp.*

Each one of the added Spirulina aqueous extracts provides different positive effects on activity. The highest biological activity was recorded by the aqueous extract from 7-days and 30-days infiltrated culture, with absent growth for B. cereus and very weak to weak growth for Klebsiella sp. compared to the control dishes. For 30-days filtrated aqueous extract, the weaker antibacterial activity was observed, where the growth of *B. cereus* and *Klebsiella sp*. was medium. The growth inhibition was less on Gram negative bacteria (*Klebsiella sp.)* Comparing to gram positive bacteria (*B. cereus).*

The presence of antibacterial activity was supported by active compounds contained in the Spirulina aqueous extracts. Amri et al., (2017) reported that Spirulina culture contain phenolics, flavonoids, steroids, terpenes. The content of active compounds in samples sometimes different, because they used different method for cultivation or extraction. George et al., (2017) stated that alkaloids have the ability as an antibacterial. According to Abedin et al., (2019), There are numerous reports on Spirulina that it possesses the ability to inhibit both Gram negative and Gram-positive bacteria but mostly on gram positive bacteria. El-Sheekh, (2014), Said that the antibacterial compound isolated from Spirulina was mostly active against Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), with medium inhibition on Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)*.

In recent years, antibiotic resistance of bacteria has been increasing (Pehlivan et al., 2021; Mohammed et al., 2022; Unal et al., 2022). In this context, the search for antibacterial drugs from cyanobacteria has increased worldwide. Conferring to Alyasiri (2017), Different concentrations of aqueous extracts were tested by the agar well diffusion method, against two strains of human pathogenic bacteria used in this study *Pseudomonas aeruginosa* as gram-negative bacteria; and *Staphylococcus aureus* as gram-positive bacteria, the investigation gave the same results that the antibacterial activity of Spirulina was mostly on gram-positive with moderate effect on gram-negative. Parisi et al. (2009), also found high antimicrobial activity of phenolic compounds in methanol extracts from Spirulina against *S. aureus.* The biomass of Spirulina was green, which is the dominant color of chlorophyll. According to Rajalakshmi and Banu (2016), chlorophyll has a potency of antimicrobial activity.

This latter supports our results that Spirulina contains an active compound, with the highest antibacterial activity against *B. cereus* and with moderated antibacterial activity against *Klebsiella sp.*

Antifungal activity of S. platensis

The antifungal activity of Spirulina was evaluated against three phytopathogenic fungal strains named *A. solani, Cladosporium sp.* and *F. culmorum*.

The results showed that Spirulina aqueous extract exerts an antifungal activity against the three phytopathogenic fungi (Figure 1). The 7-days suspension gave the highest effect by more than 87 and 83% of mycelium radial growth inhibition of *A. solani* after 7 days and 14 days successively and, more than 77% of mycelium radial growth inhibition of *Cladosporium sp.* after 14 days and finally, more than 90% of mycelium radial growth inhibition of *F. culmorum* after 14 days (Table 1).

The 30-days suspension and the 30-days filtrated supernatant gave also a significant inhibition of mycelium radial growth of the three tested fungi, but statistically, less than that obtained with the 7-days suspension (Table 1). The difference or slight decrease in effectiveness between the 7-day suspension and the rest of the aqueous extracts may be due to the accumulation of toxic metabolites on the one hand and/or to the depletion of mineral salts in the medium after being used by Spirulina cells which increased in number. In general, the results showed that to achieve good results in the control of phytopathogenic fungus, it is enough to use the 7-day suspension, and there is no need to wait longer.

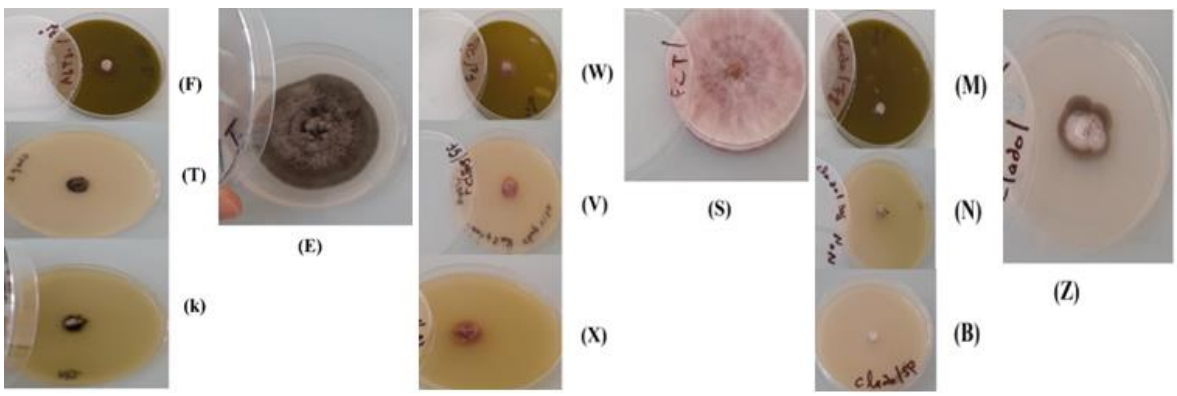

Figure 1. Results of the antifungal activity of Spirulina against three phytopathogenic fungi*;*
(W), (F), (M): 7-days suspension. (X), (B), (K): 30-days suspension. (V), (N), (T): filtrated 30days supernatant. (E): *A. solani* control dish. (W): *Cladosporium sp.* control dish. (S): *F. culmorum* control dish.

Table 1. Effect of the Spirulina aqueous extracts on the mycelium radial growth of the three tested fungi*.

Fungi Test	Incubation	*Alternaria solani*		*Cladosporium sp.*		*Fusarium culmorum*	
		ARG	IR	ARG	IR	ARG	IR
Control	7-days	6.6 ±0.10^{c}	/	2.40 ±0.15^{c}	/	8.40 ±0.00^{c}	/
Filtered 30-days supernatant	7-days	1.36 ±0.29^{b}	79.24^{a}	0.73 ±0.15^{b}	69.58^{a}	1.20 ±0.10^{b}	85.71^{a}
30-days suspension	7-days	1.30 ±0.10^{b}	80.30^{a}	0.73 ±0.12^{b}	69.58^{a}	1.16 ±0.15^{b}	86.19^{a}
7-days suspension	7-days	0.83 ±0.21^{a}	87.42^{b}	0.53 ±0.06^{a}	77.92^{b}	0.76 ±0.15^{a}	90.95^{b}
Control	14-days	8.40 ±0.00^{c}	/	5.20 ± 0.25^{d}	/	8.40 ±0.00^{c}	/
Filtered 30-days supernatant	14-days	1.36 ±0.29^{b}	79.24^{a}	0.73 ±0.15^{b}	69.58^{a}	1.30 ±0.10^{b}	84.52^{a}
30-days suspension	14-days	1.30 ±0.10^{b}	80.30^{a}	0.73 ±0.12^{b}	69.58^{a}	1.16 ±0.15^{b}	86.19^{a}
7-days suspension	14-days	1.07 ±0.14^{a}	83.79^{b}	0.53 ±0.06^{a}	77.92^{b}	0.76 ±0.15^{a}	90.95^{b}

ARG: Average radial growth (cm); IR: Inhibition rate (%); * The values given are mean (n= 9) with standard deviation. Means in the same column followed by same letter are not significantly different at P<0.05 (Duncan Significant Difference test).

Besides, it was found that there is no significant difference between the results after 7 or 14 days of incubation, which means that the antifungal compounds produced by the S. platensis, exert a biological activity with a long effect.

Regarding the susceptibility of the tested fungi towards the three aqueous extracts of Spirulina, it was found that the *F. culmorum* was the most sensitive, followed by *A. solani*, and finally the *Cladosporium sp.*

Pagnussatt et al., (2014), Aimed in their work to identify the Pas (Periodic acid–Schiff: a staining method used to detect polysaccharides) present in a culture extract of Spirulina algae and evaluate its effect on mycelial growth rate, glucosamine level, amylase activity and mycotoxin production by four strains of two lineages of *F. graminearum*. Results showed that amendment of Potato Dextrose media with Spirulina extract (3% w/v), which was mainly composed by gallic acid, greatly reduced radial growth of fungal colonies compared to media containing a single PA and the control. Also, average reductions of 40% and 62% in the glucosamine levels and the amylase activity were observed. In general, the Spirulina extract and the PAs reduced mycotoxin concentration, with an average reduction of 68% for the trichothecene mycotoxins deoxynivalenol and nivalenol.

Motallebi (2020), Said after their investigation that Spirulina had the highest antifungal activity towered: *Aspergillus niger, Aspergillus flavus, Penicillium heruri, Fari moniliforme Helminthorporium sp., Alternaria brassicae, Saccharomyces cerevisiae, and Candida albicans*.

This latter supports our results that Spirulina aqueous extracts contain active compounds against *Alternaria solani, Cladosporium sp.*, and *Fusarium culmorum*. The presence of antifungal activity was supported by active compounds contained in the Spirulina. According to El-Sheekh et al., (2014), The antimicrobial activities of *S. platensis* could be attributed to different compounds belonging to a diverse range of chemical classes. The antimicrobial activity found in Spirulina extracts could be due to containing γ-linolenic acid, active fatty acid, and the synergetic effect of lauric and palmitoleic acid. The tested microorganisms differ significantly in relation to their susceptibility to Spirulina antimicrobial substances, *Candida albicans* was the most sensitive microorganism while Gram-positive bacteria were more sensitive than the Gram-negative bacteria. This may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative bacterial cell wall is a multilayered structure bounded by an outer cell membrane and or due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism.

Conclusion

This work responds to an objective of cultivate Spirulina in modified media and evaluating the antimicrobial activity as against some pathogenic bacteria and fungi, a functional duality which makes it an ingredient of great interest for industry.

Spirulina was cultivated successfully in a modified medium and gave a yield of more than 100%. The aqueous extract of the Spirulina strain used in the present investigation showed antibacterial activity against the two pathogenic bacteria strains (*B. cereus and Klebsiella sp.)*, the investigation concludes that the antibacterial activity of S. platensis was mostly on gram-positive bacteria and with moderate effect on gram-negative bacteria.

On the other hand, the investigation concludes that the aqueous extract of the Spirulina strain has highly antifungal activity against the three tested phytopathogenic fungi (*A. solani, Cladosporium sp, F. culmorum*).

The results of his analysis proved that cyanobacteria could be a good source for production of antibacterial agents which could be effective when compared with current antibacterial compounds.

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