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Bibliographic review

Algae and their uses in agriculture. An update

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ABSTRACT

The need for sustainable agriculture and consumers of organic products have increased worldwide in recent years. For this reason, the increase in the use of biological products is one of the challenges of modern agriculture. The use of algae is one of the most viable options to use for these purposes. Algae are photosynthetic organisms of simple organization that live in water or in very humid environments. *Spirulina* is a type of green-blue microalgae, belonging to the genus *Arthrospira*, which is cultivated in many parts of the world and has a great interest in the field of biotechnology, due to its high nutritional value. With this bibliographic review it is proposed to give an overview and updated on the algae, its classification, its composition, extraction methods and characterization; as well as its uses in agriculture, emphasizing Spirulina for being an algae reproduced in Cuba, more than two decades ago, for cosmetic and pharmaceutical purposes; however, very little used for agricultural purposes.

Key words: biactive products, cyanobacteria, plants

INTRODUCTION

The inadequate use of chemical products in agriculture has caused the loss of the fertile layer of the soils, has decreased their biodiversity and has eliminated the natural enemies of pests [(1)].

Today, the indisputable need to protect the environment and fight against the adverse effects caused by climate change in agriculture, has led to the resumption, with great acceptance, of the use of plant extracts and algae, to increase the agricultural yields and for the prevention and treatment of plant diseases. These extracts are biodegradable products with low or no toxicity for animals and humans [(2,3)].

Algae, mostly belonging to the protist kingdom, are photosynthesizing organisms of simple organization, which live in water or in very humid environments. Prokaryotic cell cyanobacteria are also included in this group [(4,5)].

When talking about the use of algae as fertilizer, we must go back to the 19th century, when the coastal inhabitants collected the large brown algae carried by the tide, placed them on their land and observed the beneficial effect of these organisms on plants and the agricultural soil [(6)].

Since the 1950s, the use of algae has been replaced by extracts made from different species of macroalgae. Currently, these extracts have gained acceptance as "plant biostimulators". They induce physiological responses in plants, such as promoting plant growth, improving flowering and yield, stimulating the quality and nutritional content of the edible product, as well as prolonging shelf life. Furthermore, applications of different types of extracts have stimulated plants' tolerance to a wide range of abiotic stress [(1)].

On the other hand, green algae and cyanobacteria are involved in the production of metabolites such as plant hormones, polysaccharides, antimicrobial compounds, among others, which play an important role in plant physiology and in the proliferation of microbial communities in the soil [(7)].

Within the group of cyanobacteria is Spirulina (*Arthrospira platensis*), a cyanobacterium widely used in Cuba in the pharmaceutical and nutritional field, but little exploited in agriculture. However, in the rest of the world its use has been intensifying in the agricultural field thanks to the effects it has on the soil and plants [(8,9)]. Hence the need to carry out research related to the application of algae and especially, of Spirulina in our agriculture, with a view to reducing the use of chemical products, which are so costly for the environment and for the country's economy.

For all the above, this bibliographic review aims to give an overview and updated on algae in general and the effects that are achieved in plants with the application of these, emphasizing *Spirulina*.

Algae classification

There are some differences regarding the classification of algae; however, in general, they can be divided into three broad categories: microalgae, macroalgae, and true vascular plants, which in turn are subdivided into different groups (Table 1) [4,5,10-13].

Table 1. Classification of algae

Type of algae	Characteristics
	Microalgae
Phylo-pyrophytes (dinoflagellates)	They are mostly single-celled, having two flagella of different length. The cell is naked or has a more or less hard cover. They present a parasitic or predatory way of life [4,5]
Phylo-Chrysophytes	Known as yellow algae, they are unicellular or multicellular organisms that gather in colonies. Its main feature is the presence of chromatophores with yellow pigments that give them a golden appearance. They are of variable morphology with and without flagella and in some cases they are by rhizopods moved. They always reproduce vegetatively [8,9] of a more or less hard cover. They present a parasitic or predatory way of life [4,5]
Philo-euglenophytes	Algae with a very simple structure, the most significant characteristic of which is the presence of a photosensitive pigment stain. They have one or two flagella, which allows them to change their shape and multiply by longitudinal division [4,8,9].
Phylo-bacillaryophytes (diatoms)	They are the well-known diatoms. They are solitary forms that form star colonies [4,5].
Cyanophyceae	Known as blue-green algae (cyanobacteria), they are a type of photosynthesizing bacteria. They can resist extreme conditions of salinity, temperature and pH, because they produce mucilaginous envelopes that isolate them from the external environment when sudden changes occur [8,9]
	Macroalgae
Chlorophytes	Known as green algae, they are unicellular or multicellular organisms of highly variable shapes. Most microscopic species are native to freshwater; although there are numerous marine groups that reach large sizes. They multiply by cell division sexually or by the fusion of two gametes of different sizes [8-11]).
Pheophytes	Algae that reach sizes up to 100 m. Although they have chlorophylls, brown pigments hide them, so they have brown or brown coloration. These algae are typical of salt water, living very few in fresh water [8-11]. This group of algae is the most widely used in agriculture, with *Ascophyllum nodosum* being among the most widely used in the group for these purposes [11-15].
Rhodophytes	They are as red algae known, with lengths ranging from a few centimeters to a meter or so and comprise typical species of deep-sea marine waters, areas where other species cannot survive due to the lack of light. They are red, although they do not always have this color, sometimes they are purple, or even brownish red, despite this, they have chlorophyll. They reproduce sexually and asexually and have complicated cycles of alternation of generations [8-11].
	True vascular plants
	The true vascular or carophyte plants are very complex algae, mostly green in color, frequent on the banks of rivers and lakes, which reproduce sexually or vegetatively [8,9]

Chemical composition of algae

The chemical composition of algae, like those of plants, is closely related to their location and the conditions of the place where they grow, strongly depending on the availability of nutrients, light, salinity, depth, presence of freshwater currents and of course, contamination or heavy metal content of the water [(2)].

In algae, phytohormones and growth regulators have been identified (cytokinins, auxins, gibberellins, betaines, abscisic acid and brassinosteroids) [(15,17-22)], matrix and reserve polysaccharides (alginates, carrageenans, agar, ulvans, mucopolysaccharides and its oligosaccharides, fucoidane, laminaran, starch, and fluroid) [(1,7,22-24)]. Also as oligosaccharides, biotoxins, and antioxidant compounds (polyphenols, bromophenols, flavonoids, fluoroglucinol polymers, coumarins, flavonones, fluorothanins, protoanthocyanins, polyhalogenated diterpenes and monoterpenes, halogenated ketones and isoprenoid compounds) [(7)], chlorophylls and carotenes [(24,25)], xanthophylls [(24)]. Minerals as (iron, calcium, magnesium, phosphorus, iodine, nitrogen, potassium, barium, boron, cobalt, copper, magnesium, manganese, molybdenum, nickel and zinc), organic matter [(1,13,14,16,19,20,24,26)], mannitol [(16-18)], vitamins, amino acids and proteins [(1,2,12,13,17,19,20,24,25,27)] , alginic acids, fulvic acids and other organic acids (palmitic, butyric, oleic, linoleic [(2,16,19,27,28),] enzymes [(18,19)], sterol and fucosterol [(16)]).

A rich composition that algae possess is responsible for the beneficial effects that its application causes in plants, due to the role that many of these compounds play in their various physiological processes.

Extraction methods of the active principles of algae

To the extent that the processes from collection to extraction of the active ingredients are well adjusted, the results obtained in the field will be the best. In general, most of the extractive processes must include cell disruption to release the components of interest to the extract [(3)].

Processes may include alkali extraction [(1,15,23)], acid extraction [(1,15)], suspension cell rupture [(1,15)], enzyme digestion [(3)], high pressure water extraction [(23 , 29)], extraction with chemical solvents [(24,30)], assisted extraction with microwaves [(23,29,30)] and extraction with supercritical fluids (CO_2) [(23,29)]. Sometimes, simply, a drying followed by a spray and the powder is used to be to the ground applied. Many of these processes are carried out in most cases using low temperatures so as not to damage any metabolite [(1,15)].

Next, the extraction processes that have been most used will be described.

Extraction with alkalis

This method was developed in the 1940s and consists of the use of a base (generally potassium hydroxide), together with the application of heat. The algae used are dried at high temperatures (>100 ºC) to facilitate storage and the product obtained generally has a high pH; all this leads to a denaturation of active ingredients that result in a drastic loss of their properties [(1,15,16,23)]. This means that although this method was one of the most widely used, it is not one of the most feasible to obtain extracts with a large number of benefits.

Extraction with chemical solvents

In this method, a set of chemical solvents with different polarities are used for the extraction of their active ingredients, the most used being water and hydroalcoholic solutions and high temperatures are not used ([19,21-23,25,26,29)]. The fact of not using high temperatures, or chemical solvents that drastically affect the pH, makes this one of the preferred methods since the properties of the active principles of the algae are not affected.

Extraction with supercritical fluids (CO_2)

This method does not apply either chemical solvents or high temperatures. The raw material used has to be fresh, so the production plants have to be close to the coast. In this method, the algae is to very small particles crushed and it is to high pressure subjected, to promote the extraction of the active ingredients. Since no high temperatures are applied at any stage of the process and chemical solvents are not used either, the active principles are conserved and the pH is maintained at its physiological level of approximately 4.5 [(23,29)].

The extraction process chosen is key to obtaining a product with the composition necessary to achieve the desired effects [(1,3)] and they are chosen depending on the composition required. For example, to obtain an extract rich in auxins, alkali extraction is generally used, microwave assisted extraction has been used to obtain an extract rich in polysaccharides [(30)] and if this is combined with extraction with water at high pressures, an extract rich in fucoidans is obtained. Extraction with 70 % ethanol allows obtaining an extract rich in cytokinins, while using 85 % methanol an extract rich in gibberellins is obtained and using the extraction of supercritical fluids, extracts rich in lipids, volatile metabolites, pigments, are obtained antioxidants, carotenoids, chlorophylls, vitamin E and linoleic acid [(23)].

Products made from algae

With the aim of expanding the use of algae in agriculture, a wide variety of products is produced currently, including:

Chopped and powdered macroalgae

Algae biomass for these purposes generally comes from the exploitation of natural populations of *Ascophyllum*, *E. Macrocystis*, *Durvillea*, *Ecklonia*, *Fucus*, *Sargassum*, *Cystoseira* and *Laminaria*. It is (in the sun or in tobacco-type dryers) dried and chopped and/or ground to give flour. Generally, these are used close to the coastal areas [(1)].

These flours are "dusted or dissolved in water for hydroponic planting. On the other hand, they are spread to eroded or contaminated soils, slopes, crop fields, etc., in order to fix road slopes and clearings, regenerate poor soils and with toxicity problems, treating grass sports fields and planting steep meadows, among others [(1,31)].

Liquid algae extracts

In general, liquid algae extracts are used for foliar application as biofertilizers, although they are also applied to the soil. Some commercial extracts contain only macroalgae, although extracts supplemented with trace elements, fishmeal and pesticides are more abundant. Extracts from microalgae (live; eg: Agroplasma) and from cyanobacteria (dead; eg: "GA Gel of algae" and Agro-organic Mediterranean) appeared on the market in the late 90's [(31-36)].

There is a large number of commercial algae-based biostimulants, most of which are made from the *Ascophyllum nodosum* algae, examples of these products are Acadian, Fruticrop, Solu-Sea and Stimplex [(17,33)]. In addition, commercial products made from microalgae such as *Spirulina* or Chlorella exist for example, CBFERT and Naturplasma, respectively [(34)] or from the combination of both as the product known as Naturvita [(35)].

Uses and effects of algae in agricultura

The effects achieved with the algae extracts depend largely on the synergistic effect of the action of all the components, and the effect alone cannot be isolated from each of the active ingredients (37). These effects are with low concentrations of the extracts achieved, reaching proportions of 1: 1000 (15). These effects will also depend on the way in which these extracts are applied, being able to be applied directly to the soil, by foliar spraying, by pelletizing the seeds, post-harvest treatment or by the combination of some of them, the combination being soil treatment and foliar spraying the most widely used application mode (1,3,7,17-19,37). In this last combination, the soil is with some components enriched necessary to achieve adequate germination of the seeds and emergence of the plants, as well as better initial growth of the same. Then, the foliar application will benefit both the vegetative and reproductive development of the plants, which can be translated into a stimulation of the yield and a better quality of the harvest.

Among the effects of algae and their extracts are; stimulating seed germination (15,38), plant growth (1,15,18,19,24,31) and flowering and delaying senescence (2,4). On the other hand, they stimulate root growth, advance fruit ripening (4), increase plant tolerance to abiotic stress such as salinity, drought, high temperatures and frost, and have fortifying effects (2,4,15 -23).

Algae also act in the processes that trigger plant defense and immunity mechanisms (3,7,26,39), reduce nematode infestation (40) and increase resistance to fungal and bacterial diseases (41,42); as well as increases resistance to attack by mites, aphids, spider mites, whiteflies, aphids and nematodes (15). In recent studies, the potential of algae extracts has been shown to control various types of fungi, since the treated plants have increased their resistance to diseases caused by *Fusarium* sp., *Botrytis* sp. and *Alternaria* sp (7,24,43).

Several studies have indicated that when algae or their derivatives are applied to the soil, their enzymes cause or activate reversible catalytic enzymatic hydrolysis reactions; in addition, they hydrate and restructure the soil (1,17,20,24). Unlike chemical fertilizers, algae release nitrogen more slowly and are rich in macro and microelements (1,12-16); therefore they have been widely used as soil fertilizers (6,12,44). In addition, they have been used to reduce the amount of exchangeable sodium, which leads to the recovery of sodium soils (45).

It is worth noting the effect of algae on various physiological processes of plants, such as: photosynthesis (22), respiration and the mobilization of nutrients to the vegetative organs

[39,46]. Furthermore, they promote diversity and microbial action in the soil [1,9,12,17,20], thus creating an adequate environment for the radical development of plants [14,22,24].

Biofertilizers based on algae such as alga enzymes, turbo enzymes and algarrot, applied to the soil and by foliar route, to a vine plantation (*Vitis vinifera*) cv Shiraz, increased the rate of CO_2 assimilation and reduced the rate of evapotranspiration. It resulted in an increase in the efficiency of the use of water and in the improvement of the fruits [27].

On the other hand, it has been shown that the treatment of rice plants with blue-green algae increased the production of the grains. In countries such as India and Southeast Asia, where rice is the main component of food, the use of algae as natural fertilizers has been presented as a more than interesting method [47. In addition, under watering conditions, these algae provide the soil with organic matter, vitality, productivity and fertility, improves its physical and chemical properties, and soil microorganisms increase the ability to metabolize molecular nitrogen, increase the release of part of the fixed nitrogen and the solubility of insoluble phosphorus [36].

In studies carried out on maize, with lipid extracts obtained from microalgae, mineral fertilization was reduced and productivity increased [48].

In fruit trees, cereals, leafy vegetables and fruits, orchids and *Arabidopsis thaliana*, a biostimulant effect was found, defense against diseases (it acts as an elicitor and stimulates the synthesis of phytoalexins), protection against saline, hydric and thermal stress and increased performance. In citrus (applying in soil in addition to foliar application), it stimulated the availability of sugars, increased the size of the fruits and improved their quality, and increased the length and osmotic potential of the stem. [1,4,7,27].

On the other hand, organic extracts of Brazilian marine algae showed antifungal activity against anthracnose of banana and papaya [49] and aqueous and organic extracts of *Sargassum vulgare*, applied at different concentrations in potato tubers (*Solanum tuberosum* L.), showed an activity antifungal against *Pythium aphanidermatum*, where the highest activity was observed when the methanolic extract was used [26].

The brown algae *Ascophyllum nodosum* is one of the most widely used in agriculture internationally, which may be due to its rich composition of alginates, mannitol, betaines, polyphenols, oligosaccharides (laminanes and fucans), flavonoids, nutrients (nitrogen, phosphorus, potassium, calcium, iron, magnesium, zinc, sodium and sulfur) and amino acids (1,26,27,50) and the fact that this algae abounds on the sea coasts. Among the effects achieved with this alga can be mentioned:

1. The increase in the mass and size of the fruit, as well as the acceleration of the ripening phase by the application of extracts of this algae in kiwi (50).
2. The stimulation of the growth and consumption of calcium, potassium and copper of the plants, as well as the increase in the size, mass, firmness and fruit production in the vine cultivation by the foliar application of extracts of this (1,51).
3. The promotion of growth, of the content of chlorophylls, N, K, Fe, Mn and Zn in the leaves of apple plants by the foliar application of extracts of this algae (2 mL L^{-1}) together with amino acids (0, 5 mL L^{-1}). Furthermore, fruit production increased with the application of the extract alone and in combination with amino acids (52).
4. The increase in the leaf area and the content of chlorophylls, carbohydrates, nitrogen and zinc in the leaves of peach plants by foliar spray at a concentration of 4 mL L^{-1} (53).
5. The increase in the total content of phenols, total flavonoids and total isothiocyanates in two broccoli cultivars by the application of extracts of these algae (54).
6. Stimulation of germination and reduction of the emergence time of plants in the bean crop by immersing seeds in an extract of these algae at a concentration of 0.8 mL L^{-1} for 15 minutes (55).
7. The stimulation of the growth and performance of onion plants by the application of an extract of these algae with a dose of 2.5 g m^{-2} (56).
8. In contrast, algae of the order of *Corallinales* (Coralinas), when presenting their carbonate-rich composition, have been used as soil conditioners, since they correct the pH in acidic soils and in turn provide numerous trace elements (24).

As for the *Acutodesmus dimorphus* algae, the application of cell extracts to the seeds in a concentration of 0.5 g mL^{-1} increased the germination speed; while foliar application at a concentration of 3.75 g mL^{-1} increased the height of the plant and the number of branches and flowers and the mixture of 50 and 100 g with the potting soil. 22 days before transplanting stimulated significantly the growth and number of branches and flowers (57).

General characteristics of Spirulina and effects of application in agriculture

Spirulina (*Arthrospira platensis*) is a type of blue-green algae, which has a great interest in the field of biotechnology, its pharmaceutical use and as human and animal food being highly exploited, because it is cultivated in many parts of the world for its high nutritional value [25,58].

Spirulina has approximately 60 –70 % of its dry mass in proteins with high bioavailability. It is the terrestrial and aquatic organism with the highest protein content and the best aminogram and digestibility [8], reason why it is widely used as a source of amino acids for men, animals and plants. In addition, it contains essential polyunsaturated fatty acids and vitamins [25], as well as xanthines, phycobiliproteins [25,59], carbohydrates, nitrogen, phosphorus, potassium, calcium, iron, manganese and zinc [60].

It also has a high content of vitamins B12, B1, B2, B6 and E, biotin, pantothenic acid, folic acid, inositol and niacin [39]. Also, great richness in α- and ß-carotenes [25,61], phycocyanin, considerable amounts of α-linolenic acid (polyunsaturated fatty acid with different beneficial effects), a high concentration of phytohormones, trace elements, antioxidants and polysaccharides, therefore, it is an excellent biological complement [62]. Furthermore, chlorophyll a, xanthophylls and lipids have been in these algae identified [24].

Application of Spirulina and its extracts in agriculture

With the evolution of sustainable agriculture, the use of Spirulina has been increasing for these purposes. It has been shown to activate the immune system of plants, generating higher productions, of higher quality and more resistant to diseases and environmental stress, as well as greater germination and rooting when applied to the soil. When comparing a Spirulina-based fertilizer with a chemical fertilizer, some authors have found that although it has a lower NPK content. The fertilizer based on these algae stimulates the growth of crops in a similar way to the chemical fertilizer, because it has higher amounts of other elements (calcium, iron, manganese, zinc and selenium) that help moderate the amounts of nutrients required by plants [60]. Furthermore, phenolic extracts of Spirulina have been shown to exhibit antifungal activity against *Fusarium graminearum* [61].

Various authors have reported the effects that the application of Spirulina has caused in different plant species. Thus, in *Amaranthus gangeticus*, it has been found that the imbibition of the seeds and the foliar application of Spirulina extracts increased the protein (62) and iron levels in the plants (63). Similarly, it was reported that the imbibition of *Phaseolus aureus* and *Solanum lycopersicum* L. seeds in extracts of this species, increased Zn levels in plants (64).

In the *Solanum melongena* L. species, the application of a commercial fertilizer based on Spirulina increased the yield of the plants without affecting the foliar levels of N, P, K and Na or its quality indicators (65). Foliar application of a similar fertilizer maintained the quality indicators of the *Lactuca sativa* L. plants after harvest, preserving the content of soluble solids, titratable acidity, vitamin C, chlorophyll a and total chlorophylls (66).

In beans, the foliar application of an aqueous extract stimulated growth, chlorophyll, nitrogen, phosphorus and potassium concentrations; as well as the quantity and quality of the seeds (67).

The effects of combining Spirulina extracts with other biofertilizers have also been reported. For example, in plants of *Origanum vulgare* L., the combination of Spirulina extract with a biofertilizer based on bacteria significantly stimulated the growth, performance and production of essential oils (68). While in *Solanum tuberosum* L. plants the combination of *Chlorella vulgaris* and *Arthrospira platensis* extracts improved the vital conditions of potato and hybrid seed production in Hadúszoboszló areas in India (45).

The effects shown are closely related to the chemical composition of Spirulina, which was previously described. It is known that the active ingredients that it possesses such as proteins, amino acids and carbohydrates exert a great influence on the growth and development of plants, the macro and microelements content, stimulates plant nutrition and is used as a bio-fortifying agent in some crops. Spirulina also has growth regulators and antioxidants that are capable of increasing plants' tolerance to environmental stress conditions, among others.

In Cuba, Spirulina has been widely used for pharmaceutical, cosmetic and nutritional purposes. These microalgae has not been practically used in agriculture, despite the fact that its chemical composition and the influence that its application could have on plant growth and development are known, as well as the benefit it can cause in soils for the quantity and quality of nutrients it has. It is known about some specific investigations carried out with some biofertilizers based on Spirulina, such as **CBFERT** (34), as well as a more recent biostimulant based on Spirulina and Vinasse (Spirufert, product in the registration phase), which is being evaluating its foliar use in some crops (unpublished data). Currently, some studies are being to optimize the doses carried out, timing and mode of application of this biostimulant; as well as its interaction with other biostimulants produced in Cuba, with a view to expanding its use in agriculture.

In addition, it would be very beneficial for Cuban agriculture to be able to have extracts of this cyanobacterium and other marine algae, which can be applied to both the soil and seeds and plants, to not only stimulate growth and yield, but also improve the harvest quality and the physical, chemical and biological properties of the soil.

CONCLUSIONS

- The use of algae offers a great benefit for a sustainable and more environmentally friendly agriculture; since they are natural products, which have a variety of substances that stimulate the growth and yield of crops; they favor the microbial activity of the soil and improve the absorption of nutrients by the roots. In addition, they give plants an effective resistance to abiotic stress, because they contain substances with a high antioxidant power.
- Taking into account all the results presented in this review about the effects of algae in agriculture, the need to increase the sustainability of agricultural production and stimulate the resilience of crops to the adverse effects associated with climate change; it is necessary, in Cuba, to accelerate research related to the application of algae and especially of Spirulina in agriculture.

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