



BIOFERTILIZATION WITH CHLOROPHYTA AND CYANOPHYTA: AN ALTERNATIVE FOR ORGANIC FOOD PRODUCTION

Biofertilización con clorofitas y cianofitas: una alternativa para la producción de alimentos orgánicos

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ABSTRACT

Chlorophyta and Cyanophyta are photosynthetic organisms characterized by their biochemical plasticity, which has allowed them to develop in different environments and have a faster growth rate than plants. Depending on the species and environmental conditions, these organisms can produce nitrogenous enzymes, for atmospheric nitrogen fixation; phosphatases, that solubilize phosphorus; phytohormones, that promote plant growth; and hygroscopic polysaccharides, that prevent erosion and improve soil characteristics. In this sense, the aim of this review was to analyze the available information on the use of Chlorophyta and Cyanophyta as biofertilizers and their potential application in organic food production. Multiple studies and researches were found demonstrating the advantages of these microorganisms when being used to improve plants productivity, and also at the same time, leading to sustainable agriculture that is respectful to the environment. However, their high production cost has become a limiting factor for their commercialization.

Keywords: Biofertilizers, cyanobacteria, green algae, organic agriculture.

RESUMEN

Clorófitas y cianófitas son organismos fotosintéticos que se caracterizan por su plasticidad bioquímica, lo que les ha permitido desarrollarse en diferentes ambientes y tener una tasa de crecimiento más rápida que las plantas. Dependiendo de la especie y las condiciones ambientales, estos organismos pueden producir enzimas nitrogenadas para la fijación del nitrógeno atmosférico; fosfatasas que solubilizan el fósforo; fitohormonas que promueven el crecimiento de las plantas; y polisacáridos higroscópicos que evitan la erosión y mejoran las características del suelo. En este sentido, el objetivo de esta revisión fue analizar la información disponible sobre el uso de cianófitas y clorófitas como biofertilizantes, y su posible aplicación en la producción de alimentos orgánicos. Múltiples estudios e investigaciones fueron encontrados demostrando las ventajas del uso de estos microorganismos para mejorar la productividad de las plantas, y que a su vez conducen a una agricultura sostenible respetuosa con el medio ambiente. Sin embargo, su alto costo de producción se ha convertido en un factor limitante para su comercialización.

Palabras clave: Agricultura orgánica, algas verdes, biofertilizantes, cianobacterias.

INTRODUCTION

Chlorophytes and cyanophytes are photosynthetic organisms characterized by their biochemical plasticity, which has allowed them to develop in different environments and have a faster growth rate than plants. Both chlorophytes and cyanophytes, do not have tissue-specific biochemistry activity, meaning that each cell produces all the necessary substances for photosynthesis, development, and reproduction. Therefore, interest in the biotechnological potential of these organisms has increased, given their adaptability to large scale production technologies. Some of these microalgae main biotechnological applications are high nutritional value and healthy food production, hydrogen production as biofuel, ecosystem restoration, and crops biofertilization (Guedes *et al.*, 2011; Berg *et al.*, 2014; Nyberg *et al.*, 2015; Verseux *et al.*, 2016; Chamizo *et al.*, 2018).

According to the species and environmental conditions, these organisms can produce nitrogenous enzymes, for atmospheric nitrogen fixation; phosphatases, for phosphorus solubilization; phytohormones similar to cytokinins for plant growth, such as iso-pentenyladenine and zeatin; and, hygroscopic polysaccharides that prevent erosion and improve soil characteristics (Osman *et al.*, 2010; Lu and Xu, 2015; de Siqueira Castro *et al.*, 2017). These features have awakened interest in chlorophytes and cyanophytes investigation as alternatives for organic crops fertilization. Multiple laboratory and field experiments had evaluated these organisms' application in crops like rice, corn, wheat, tomatoes, and others, especially in countries with limited access to chemical fertilizers (Coppens *et al.*, 2016; Renuka *et al.*, 2016; Chittapun *et al.*, 2018; Dineshkumar *et al.*, 2018; Dineshkumar *et al.*, 2019). Thus, this review aims to analyze the available information on chlorophytes, and cyanophytes use as biofertilizers for organic food production, being an alternative to products obtained from chemical syntheses, such as conventional fertilizers and pesticides.

BIOFERTILIZATION WITH CHLOROPHYTA AND CYANOPHYTA AS AN ALTERNATIVE IN AGRICULTURE

Soil fertilization is a relevant and limiting factor for crops growth and productivity due to crops that extract large amounts of nutrients, and agricultural practices that decrease organic matter (OM) content, which is essential for soil structure, soil biodiversity, buffer capacity, thermal conductivity and soil fertility (Nain *et al.*, 2010; Lin *et al.*, 2013); and can also affect soil's water retention (Lehmann and Kleber, 2015; Schlatter *et al.*, 2017). Research has shown that soil processes related to phosphorus, carbon and nitrogen cycles, and also soil quality indicators such as arbuscular mycorrhizal fungi, community-level physiological profiles, alkaline phosphatase, and dehydrogenase activities, are very sensitive to the indiscriminate use of chemical

fertilizers causing serious consequences such as lower nutritional quality, soil structure degradation, and change in the physical, chemical and biological soil conditions as its microbiota is altered (Souza *et al.*, 2016; Malik *et al.*, 2017; Nivelles *et al.*, 2018).

As an alternative to commercial fertilizers, a series of biofertilization techniques had been developed, like composting use, organic and biological fertilizers, all of them aiming to promote sustainable agricultural practices favorable to the environment and that generate higher nutritional quality products (Vandana *et al.*, 2017; Helmy, 2018). Biofertilizers are substances that contain alive microorganisms and improve the plant's development. The difference between these products and chemical fertilizers consist in the biofertilizer's gradual nutrients and phytostimulants supply, that improves soil characteristics and provides optimal plant growth (Carvajal-Muñoz and Carmona-Garcia, 2012; Mosa *et al.*, 2015). Biofertilization stimulates soil properties by increasing OM content, facilitating cation-exchange capacity (CEC), raising water retention, promoting aggregates formation, and improving soil's buffering capacity, through the presence of polysaccharides and mucilaginous substances that provide the cohesiveness for binding soil mineral particles and thereby help in soil structure formation (Carvajal-Muñoz and Carmona-Garcia, 2012; Vitousek *et al.*, 2013; Ghosh, 2018).

Owing to its ion retention capacity, OM preserves nitrogen, and phosphorus by diminishing these nutrients leaching, and favors microorganisms root colonization and prevents phytopathogens development (Lehman *et al.*, 2015). Therefore, microbial inoculants have been used in sustainable agriculture to maintain high productivity and crop quality with lower costs compared to chemical fertilizers (Sarma *et al.*, 2015; Li *et al.*, 2017; Vandana *et al.*, 2017).

Furthermore, biofertilizers are an alternative to improve agricultural productivity in erosion susceptible soil, which can be caused by the lack of OM, due to its particle aggregating effect (Cotrufo *et al.*, 2015; Lehman *et al.*, 2015). A strategy to increase OM and soil nutrient levels consist in adding microorganisms that produce mucilaginous polysaccharides (intracellular and extracellular), which are hygroscopic and have adhesive properties that act as soil particle aggregating agents, thus, increasing soil's porosity and improving its structure (Colica *et al.*, 2014; Rossi and de Philippis, 2015). These effects in the soil can last for several months, and afterward, during the degradation of polysaccharides, plants receive those nutrients gradually (Park *et al.*, 2017; Li *et al.*, 2018). Some chlorophytes and most cyanophytes have a high production of these polysaccharides and mucilage, as they are components of the cell wall (Ghosh, 2018). Recent research uncovered their many possible applications not only in agriculture but also in biomedicine due to their antibacterial, antiviral, antifungal, and anticoagulant activity (Guo *et al.*, 2015; Berri *et al.*, 2016; Faggio *et al.*, 2016).

Chlorophytes and cyanophytes are fast-growing photoautotrophic organisms with the ability to adapt to different environmental conditions, which allows them to be established in small areas and regions non-suitable for crops (Sorochkina *et al.*, 2018). Cyanophytes are primary colonizers during soil's successional processes, and can even grow over volcanic ashes (Kerfahi *et al.*, 2017). It is estimated that there are between 22 000 and 26 000 species, of which only a few are used for commercial purposes, including *Spirulina*, *Chlorella*, *Nostoc*, *Anabaena*, *Haematococcus*, *Dunaliella*, *Botryococcus*, *Porphyridium*, *Scenedesmus*, *Nitzschia*, *Isochrysis*, *Schyzochytrium*, and *Phaeodactylum* (Lu and Xu, 2015; Galarza *et al.*, 2016; Hagemann and Hess, 2018).

In chlorophytes and cyanophytes, mucilage synthesis is considered a strategy to tolerate various types of environmental stress caused by dehydration, mechanical damage, UV radiation, and high temperatures. Mucilage acts as a metal ions chelator, approaching to plant's cell wall

and facilitating its absorption. Also, it favors the specialized microbial interactions between different microenvironments and biomineralization processes (de los Ríos *et al.*, 2015; Fimbres-Olivarria *et al.*, 2018). Cyanophytes can improve desert soil characteristics due to their mucilaginous polysaccharides and other metabolites production, that increase soil levels of nitrogen and carbon, and capture water from the atmosphere, allowing the development of vascular plants like *Coleogyne ramosissima*, *Stipa hymenoides*, *Streptanthella longirostris*, *Lepidium montanum* var. *jonesii*, *Agriophyllum squarrosum*, *Agropyron mongolicum*, *Artemisia ordosia*, and *Elymus dahuricus* (Wierzchos *et al.*, 2015; Rasuk *et al.*, 2016).

The use of chlorophytes and cyanophytes as biofertilizers is called “algalization”, a term developed by G.S. Venkataraman in the 1970s. Research on this topic has focused on the use of chlorophytes and cyanophytes to offer nitrogen in crops. However, as seen in Figure 1,

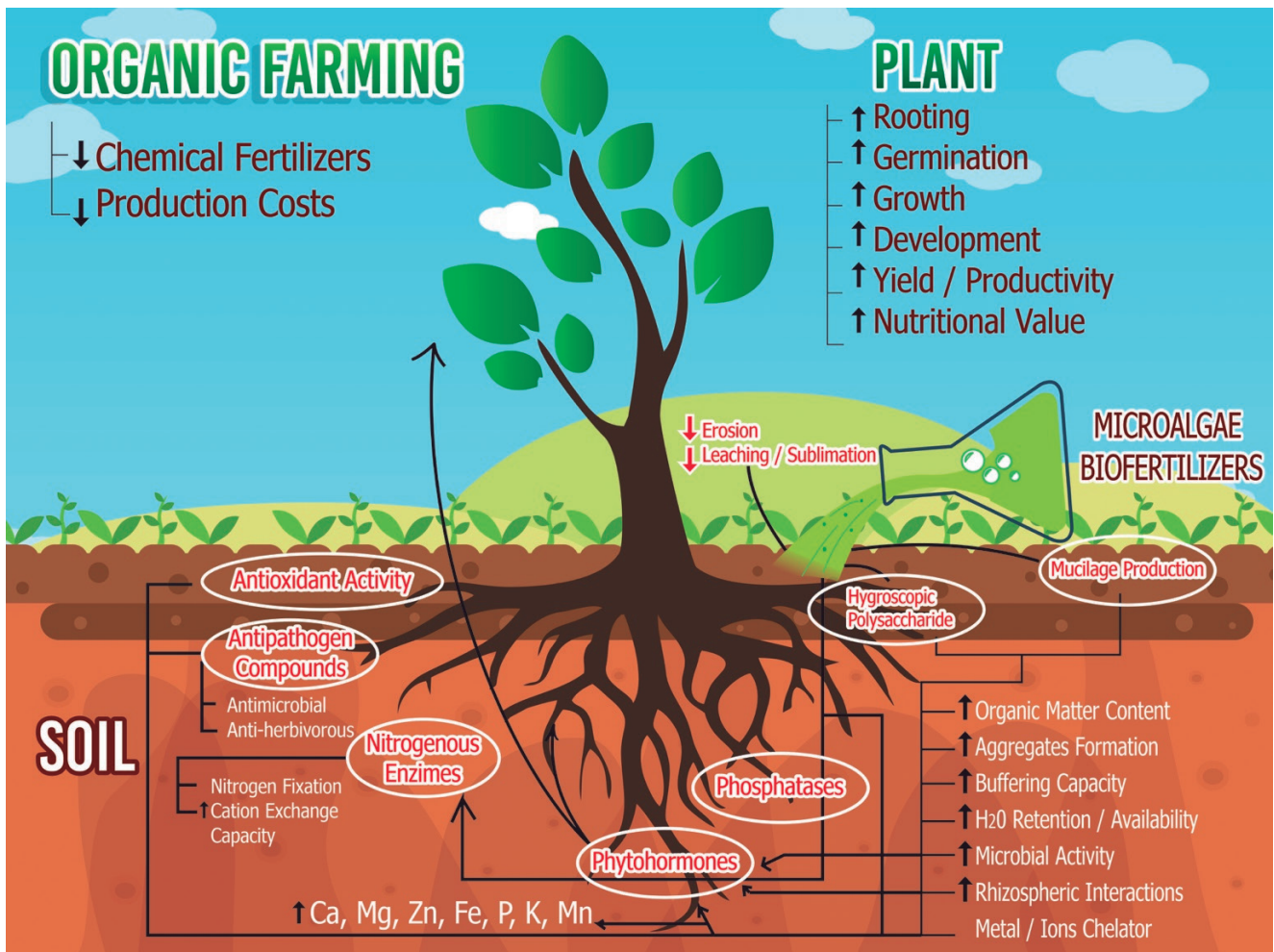


Figure 1. Effects of cyanophytes and chlorophytes as microalgae biofertilizers. In soil, they produce mucilage, hygroscopic polysaccharides, phosphatases, phytohormones, nitrogenous enzymes, antipathogen, and antioxidant compounds that enhance nutrient uptake and growth and protect the roots. In the plant, there is an improvement in rooting, germination, growth and development, yield and nutritional value of the final consumption product.

besides nitrogen fixation they can also improve soil physical properties, increase the nutrients available for plants, and produce substances that promote plant development (Dash *et al.*, 2016; Rossi *et al.*, 2017; Helmy, 2018). Indeed, the presence of cytokines had been described in cyanophytes like *Calothrix* sp, *Nostoc* sp, and *Phormidium animale* (Frébortová *et al.*, 2017). This type of phytohormones is responsible for increasing productivity in crops fertilized with cyanophytes, which do not necessarily correspond to higher nitrogen soil levels, but a major cell division and differentiation caused by the stimulating of plant growth (Haque *et al.*, 2017).

BIOFERTILIZATION WITH CYANOPHYTES

Several investigations have shown the viability of cyanophytes use as biofertilizers in plants (See Table 1), especially in rice, providing nitrogen through N₂ atmospheric fixation, and large amounts of polysaccharides that immobilize water on the soil surface during the dry season (Iyovo *et al.*, 2010). This retained water remains available to plants, prolonging their growing season and significantly increasing their yields (Odjadjare *et al.*, 2017). Moreover, polysaccharides can also prevent wind erosion during the dry season, due to their aggregation effect on the surface soil particles (Dash *et al.*, 2016; Padhy *et al.*, 2016; Chittapun *et al.*, 2018).

In rice crop, the nitrogen balance depends on microalgae as they participate in nitrogen fixation and mineralization. For this reason, paddy rice crops are fertilized with cyanophytes mixtures in numerous countries such as China, India, Indonesia (Shahane *et al.*, 2015; Rossi *et al.*, 2017; Yao *et al.*, 2018), Spain and some of South America (Ranjan *et al.*, 2016; Jhala *et al.*, 2017). In rice, *Nostoc* and *Anabaena* strains, both nitrogen fixers, are predominant, reducing the use of chemical fertilizers up to 15 %, and contributing to the biological fixation of approximately 20-30 kg of nitrogen per hectare, in each growing season (Chittapun *et al.*, 2018; Singh *et al.*, 2018).

Biofertilization with cyanophytes has also been evaluated in other crops such as wheat (Li *et al.*, 2017; Di Salvo *et al.*, 2018), legumes (Sonkoly *et al.*, 2017; Muñoz-Rojas *et al.*, 2018), and green peas, where cyanophytes from *Nostoc* genus stimulated plant growth and increased seed germination, reducing the use of chemical fertilizers by 50 % (Osman *et al.*, 2010). Furthermore, Maqubela *et al.* (2009) evaluated fertilization in maize also with *Nostoc* sp, finding that plants had between 40-49 % more dry weight and 14-23 % more N in their tissue. Similarly, Grzesik and Romanowska-Duda (2014) biofertilized maize, using cyanophytes strains from *Microcystis aeruginosa* and *Anabaena* sp, finding not only improvements in biomass and nutrient uptake, but also in percentage, dynamics, and mean time of germination. The authors associated these events with a high concentration of different bioactive compounds included in Cyanobacteria

that stimulate physiological pathways inside the plant, like the assimilation of atmospheric nitrogen, increases in chlorophyll content in leaves, activity of net photosynthesis, transpiration, stomatal conductance, intercellular CO₂ concentration, activity of acid and alkaline phosphatase, RNase, total dehydrogenase, and a decrease in electrolyte leakage from leaves, which indicates lower permeability of cytomembranes under the application of Cyanobacteria (Grzesik and Romanowska-Duda, 2014). Later, the same authors confirmed those finding using willow plants (Grzesik *et al.*, 2017).

Overall, several authors had concluded that the species of Cyanophyta mentioned above can be an alternative replacement for chemical fertilization in different crops and that it is compatible with organic agriculture, including horticulture (Chamizo *et al.*, 2018). Additionally, it has been observed that Cyanophyta also stimulates microbial activity in soils damaged by fire and in arid and semi-arid soils, reducing water and wind erosion (Nisha *et al.*, 2018).

BIOFERTILIZATION WITH CHLOROPHYTES

Cyanophytes use is widely known, but its applications are limited by some species possibility of producing toxic compounds (Kaushik *et al.*, 2019). Since chlorophytes do not have this disadvantage, they offer interesting possibilities given their ease and speed of growth, as well as, for their nutrient content that includes Ca, Mg, Zn, Fe, P, K and Mn (Carvajal-Muñoz and Carmona-Garcia, 2012).

Moreover, Chlorophytes can produce polysaccharides and some phytohormones, which could favor soils recovery, improve nutrient content, and enhance plants growth (Grzesik *et al.*, 2017; Schreiber *et al.*, 2018). For instance, irrigation of wheat and rice crops with chlorophytes suspensions, as well as the immersion of seeds in those suspensions, had shown numerous benefits, such as an increase in germination and productivity rates (Galarza *et al.*, 2016; Huang *et al.*, 2016; da Silva Ferreira and Sant'Anna, 2017; de Siqueira Castro *et al.*, 2017; Dineshkumar *et al.*, 2018).

Out of all microalgae, *Chlorella* genus has been most used for biofertilization so far and was the first microalga to be cultivated (Wijffels *et al.*, 2013). *Chlorella* is a unicellular, non-mobile Chlorophyta with 2-10 µm diameter. It is known for its food potential, given its high content of proteins and other nutrients. The use of *Chlorella vulgaris*, for instance, is widely known. Eman *et al.* (2008) used extracts of *C. vulgaris* on grape cultivations by adding them in concentrations of 25 % and 100 %, causing a positive effect on productive bulbs percentage when comparing to the control treatment. Other characteristics like leaf area, stem length, and leaves and buds number, were too positively affected. Moreover, they observed a slight increase in the productivity of the vines expressed in the number of bunches, the weight of the grapes, the fruits quality, a reduction in the ripening time,

Table 1. Use of Chlorophyta and Cyanophyta as biofertilizers in different crop and non-crop plants.

Plant	Organisms	Application	Effect	Country	Reference
Williams banana (<i>Musa cavendishii</i>)	<i>Chlorella vulgaris</i> (Chlorophyta)	Spray four times during growing season at 0.0, 25, 50, 75 and 100 % concentrations.	Improvements: yield, bunch and hand weight, and chemical properties (total soluble solids % and total sugars, decrease in % of starch and total acidity).	Egypt	Eman <i>et al.</i> , 2008
Maize (<i>Zea mays</i>)	<i>Nostoc</i> sp. (Cyanophyta)	1 g dry weight/L suspension on soil (dry biomass: 6 g/m ²).	Improvements: soil's C, N and EPS content, and aggregate stability. Plant's growth and N uptake.	South Africa	Maqubela <i>et al.</i> , 2009
Pea (<i>Pisum sativa</i>)	<i>Nostoc entophyllum</i> (Cyanophyta)	Fresh weights: 0.5, 1 and 1.5 g dissolved in 100 mL of distilled water each (OD: 0.95 at 700 nm) added to the soil (3 Kg soil/pot).	Improvements: germination percentage, growth parameters (root depth, shoot length, dry weight, leaf area, and number), pigments content (chlorophyll a and b, carotenoid), carbohydrate, total N and P, and protease and amylase activities.	Egypt	Osman <i>et al.</i> , 2010
	<i>Oscillatoria angustissima</i> (Cyanophyta)				
Maize (<i>Zea mays</i>)	<i>Microcystis aeruginosa</i> (Cyanophyta)	Monocultures suspended in water applied to grains up to 35 % for 2 days, and continuous moistening of the substrate (filter papers).	Improvements: germination percentage, dynamics, and mean time of germination and accelerated growth of seedlings (faster elongation of roots and leaves and enlarged fresh and dry biomass). Increase in chlorophyll content, the activity of net photosynthesis, and others.	Poland	Grzesik and Romanowska-Duda, 2014
	<i>Anabaena</i> sp. (Cyanophyta)				
	<i>Chlorella</i> sp. (Chlorophyta)				
Cucumber (<i>C. sativus</i>) Eggplant (<i>S. melongena</i>) Rice (<i>O. sativa</i>) Lettuce (<i>L. sativa</i>).	<i>Chlorella vulgaris</i> (Chlorophyta) <i>Chlorella pyrenoidosa</i> (Chlorophyta)	Seed watered with 2 ml of <i>C. vulgaris</i> solution (289×10 ⁴ /ml) or <i>C. pyrenoidosa</i> solution (11.8×10 ⁴ /ml) twice a day.	Improvements: healthier seedlings with the enhanced root system. Seedlings of cucumber and eggplants had greener and bigger leaves. Cucumber seedlings were disease resistant. Higher chlorophyll a and b content, except in rice.	Dubai, UAE	Elhafiz <i>et al.</i> , 2015
Wheat (<i>Triticum aestivum</i>)	<i>Chlorella</i> sp., <i>Scenedesmus</i> sp., <i>Chlorococcum</i> sp. (Chl), <i>Chroococcus</i> sp. (Cya)	Dry biomass (20 µg chlorophyll/g vermiculite: compost) with water. 50 g of each formulation (MC1 and MC2) was mixed with 6 Kg of soil.	Improvements: higher values of available N, P, and K in roots, shoots, and grains, and better nitrogen-fixing potential. Microbial biomass carbon significantly enhanced. Increase in plant dry and spike weight.	India	Renuka <i>et al.</i> , 2017
	<i>Phormidium</i> sp., <i>Anabaena</i> sp., <i>Westiellopsis</i> sp., <i>Fischerella</i> sp. (Cya)				
	<i>Aphanothece</i> sp. (Cyanophyta)				
	<i>Gloeotrichia</i> sp. (Cyanophyta)				
	<i>Ulothrix</i> sp. (Chlorophyta)				
Rice (<i>Oryza sativa</i>)		10 Kg dry weight/ha.	Improvements: grain yield and panicle number.	India	Dash <i>et al.</i> , 2016
Tomato (<i>Solanum lycopersicum</i>)	<i>Klebsormidium</i> sp. (Chlorophyta)	Blended with the organic growing medium.	Improvements: fresh weight, sugar and carotenoid content of fruits.	Belgium	Coppens <i>et al.</i> , 2016
	<i>Chlorella vulgaris</i> (Chlorophyta)	Foliar application of suspension: 10 L/ha.	Improvements: greener, larger and healthier leaves, K and Ca content, apple weight and size.	Hungary	Nagy, 2016
Rice (<i>Oryza sativa</i>)	Commercial packets of N ₂ -fixing of Cyanophyta	1 Kg/m ²	Improvements: growth (shoot length, leaf area and plant dry weight), crop yield, leaf metabolic activities (chlorophyll a, catalase activity and protein /carbohydrate ratio), and soil properties (silt %, N content, and amelioration of metal contents).	India	Padhy <i>et al.</i> , 2016
Willow (<i>Salix viminalis</i>)	<i>Microcystis aeruginosa</i> (Cyanophyta)	Foliar application of monocultures (2.5×10 ⁵ cells/mL) three times during vegetation season, 3-week intervals.	Improvements: plant height, total shoot length, and number, FM and DM, chlorophyll levels and intensify gas exchange. Better physiological performance and crop yields, by enriching plants with growth-promoting substances. Improvement in plant health status.	Poland	Grzesik <i>et al.</i> , 2017
	<i>Anabaena</i> sp. (Cyanophyta)				
	<i>Chlorella</i> sp. (Chlorophyta)				

(Continued)

Table 1. Use of Chlorophyta and Cyanophyta as biofertilizers in different crop and non-crop plants.

Plant	Organisms	Application	Effect	Country	Reference
Maize (<i>Zea mays</i>)	<i>Chlorella vulgaris</i> (Chlorophyta)	3 g dry powder/Kg soil before planting.	Improvements: shoot length, leaves number, dry and fresh weight, total plant length, nutrients and pigments content and germinability of the seeds produced.	India	Dineshku-mar <i>et al.</i> , 2019
	<i>Spirulina platensis</i> (Cyanophyta)				
Sugar beet (<i>Beta vulgaris</i>)	<i>Chlorella vulgaris</i> (Chlorophyta)	2 and 4 mL/L in hydroponic solution.	Improvements: root traits and expression of genes related to nutrient acquisition.	Italy	Barone <i>et al.</i> , 2018
	<i>Scenedesmus quadricauda</i> (Chlorophyta)				
Rice (<i>Oryza sativa</i>)	<i>Nostoc carmeun</i> (Cyanophyta)	6 and 12 g wet in 12 Kg of soil.	Improvements: root length, shoot length, wet weight, and dry weight of seedlings.	Thailand	Chittapun <i>et al.</i> , 2018
	<i>Nostoc commune</i> (Cyanophyta)				
Rice (<i>Oryza sativa</i>)	<i>Chlorella vulgaris</i> (Chlorophyta)	Mixed with soil by soil drench method in concentration: 25, 50, 75 and 100 %.	Improvements: plant height, leaves number, leaf area, fresh and dry weight, seed number, weight of seeds, seed weight and yield. Increase in rice yield up to 7–20.9 %.	India	Dineshku-mar <i>et al.</i> , 2018
	<i>Spirulina platensis</i> (Cyanophyta)				
Orchid (<i>Schomburgkia crispa</i>)	<i>Chlorella sorokiniana</i> (Chlorophyta)	96×10 ⁵ cells/mL used as suspension and supernatant in the culture medium.	Improvements: leaf and root length, shoot fresh and dry weigh, number of roots and leaves, pigmented (green) roots, shoot development and bud preparation for rooting.	Brazil	Pereira <i>et al.</i> , 2018
Bean (<i>Phaseolus vulgaris</i>)	<i>Chlorella</i> sp. (Chlorophyta)	Foliar application (3×10 ⁶ cells/mL) twice a week.	Improvements: pod number and size, seed and total dry weight, root length and in crop yield.	Ecuador	Maila, 2018
	<i>Scenedesmus</i> sp. (Chlorophyta)				

and also, an increase in the number of sugars and a decrease in the acidity. Also, in a vineyard, the use of *C. vulgaris* caused an increase in vegetative growth, productivity, and fruit quality (Nagy, 2016).

Similarly, extracts of *C. vulgaris* had a positive effect on banana crops, increasing quality and productivity (Hamouda and El-Ansary, 2017); and in lettuce, rice, cucumber, and eggplant, were it was tested alongside *Chlorella pyrenoidosa*, reporting good result from both species as they improved growth and metabolism parameters in all plants (Elhafiz *et al.*, 2015).

Another study conducted in Hungary by Nagy (2016), evaluated *C. vulgaris* use as foliar biofertilizer of apple plant (*Malus domestica* Borkh.). It was demonstrated that the use of this Chlorophyta as biofertilizer resulted in greener and healthier leaves. Although treatments did not have a significant effect on N, P, Mg, and micronutrients concentrations in the leaves, the use of the algal suspension did increase the K significantly and Ca leaves content. Furthermore, Pereira *et al.* (2018) found that the use of another *Chlorella* species, *C. sorokiniana* stimulated the *in vitro* rooting of the epiphytic orchid *Schomburgkia crispa*. This represents an alternative for its use as a supplement since it allows to obtain better yields than conventional culture media.

On the other hand, Barone *et al.* (2018) analyzed the effect of sulfate restriction on 53 genes and the morphology

of *Beta vulgaris* L., under the addition of the chlorophytes *C. vulgaris* and *Scenedesmus quadricauda*. Results indicated that at the morphological level, seedlings treated with chlorophytes showed significantly higher values for root traits related to soil exploration and nutrient uptake; and at a molecular level, the Chlorophyta extract positively regulates many of the evaluated genes, thus, demonstrating the biostimulating effects of microalgae.

Differently, Schreiber *et al.* (2018) estimated wheat growth (*Triticum aestivum* L.) on two nutrient-deficient substrates: “null Erde” and sand, with and without fertilization by wet and spray-dried algae, and with a chemical fertilization control. After the wheat growth, it was recorded that the plants grown in the sand were smaller, but the fertilization with the algae led to a growth that was comparable to the chemical fertilizer one. These results showed that algae biomass and its nutrients represent an alternative to support agriculture in marginal soils.

IS IT POSSIBLE TO APPLY MICROALGAE USE IN ORGANIC PRODUCTION?

Organic production abstains from the use of synthetic origin agrochemicals in crops, to avoid the ecological imbalance generated by xenobiotics; instead, applies techniques that allow sustainable agricultural production (Crowder and

Reganold, 2015; Reganold and Wachter, 2016). In organic crops, strategies are used to increase soil's organic matter content, and in this way, enrich the organic forms of nutrients, which are highly assimilable by plants and microorganisms. This allows to avoid losses by leaching and sublimation, that can occur when using conventional fertilizers (Prasanna *et al.*, 2014; Crowder and Reganold, 2015).

Organic production generally uses compost, humus, and manure as sources of plant nutrients. Nevertheless, due to its large volume, high transport costs, complex nature, and variable quality, it is difficult to establish an adequate dose, and there is always the risk of contamination by phytopathogens (Sarma *et al.*, 2015). In this sense, biofertilizers derived from chlorophytes and cyanophytes offer advantages because they are formed by living cells, which are incorporated into the soil producing *in situ* OM and phytostimulation (Osman *et al.*, 2010; Prasanna *et al.*, 2014; Singh *et al.*, 2017). Additionally, the product volumes applied are lower than those of the compost; that is, they generate lower transportation costs.

Nowadays, chlorophytes and cyanophytes production systems on a commercial scale, have a strict check that allows exhaustive quality control, a deep knowledge of the microalgal biomass derivatives biochemical composition, and agronomic evaluations that show their effectiveness. This permits for algae biofertilizers to be safe and formulated with precise doses (Stephens *et al.*, 2015; Silva *et al.*, 2016; Hoffman *et al.*, 2017).

Biofertilizer products derived from algae are obtained from biosecure species, which do not produce toxins or allergens, guaranteeing in this manner, not only the safety of the operators but also of the food fertilized with these products. Because of its biological origin, biofertilizers derived from algae do not contravene any of the assumptions of organic production, and they do represent an alternative for quality production (da Silva Ferreira and Sant'Anna, 2017; Kose *et al.*, 2017; Rossi *et al.*, 2017).

PERSPECTIVES ON APPLICATION OF MICROALGAE BIOFERTILIZATION IN COLOMBIA

In Colombia, most biofertilization techniques with algae had focused on macroalgae and seaweed at Caribbean areas (Tafur and Estrada, 2015; Tasende and Peteiro, 2015; Maila, 2018). However, the multiple technical advantages of biofertilizers derived from microalgal biomass, allow their application in crops under irrigation systems as in dry land, as there are formulations designed for crops with different moisture requirements (Carvajal-Muñoz and Carmona-Garcia, 2012; Nagy, 2016; Helmy, 2018). Nevertheless, costs per hectare of biofertilizers derived from chlorophytes and cyanophytes are much higher than those of other biofertilizers such as compost, since the cultivation of microalgae has high production costs associated with specialized infrastructure and labor to guarantee its quality (Grewe and Pulz, 2012; Renuka *et al.*, 2018).

A new generation of algae biofertilizers focuses on chlorophytes and cyanophytes extracts (without living cells) with lower costs. These products are especially focused on foliar nutrition, since they have high levels of vitamins, amino acids, and hydrolyzed form enzymes, that can be incorporated through stomas. These formulations are mainly oriented to crops with high added-value such as flowers or medicinal plants (Renuka *et al.*, 2018; Rizwan *et al.*, 2018). Colombia still needs to work on the implementation of chlorophytes and cyanophytes biofertilization alternative to improve product quality and to boost national agriculture towards adequate soil management and environmental sustainability.

CONCLUSIONS

Although application and quantities of Chlorophytes and Cyanophytes inoculum, in addition to the experimental conditions, are dissimilar in the reviewed works, an overall positive effect of these microorganisms on plant growth is established in all the research mentioned above. In this context, the main task of the scientists is to find ways to improve plants productivity, leading to sustainable agriculture that secures food production and is respectful to the environment at the same time; and it seems that the use of chlorophytes and cyanophytes as biofertilizers have a great potential to achieve this objective. Nonetheless, high production costs still represent a limitation in their commercialization, which is why the main focus of investigations from now on should be to generate new algal-based biofertilizers that focus on chlorophytes and cyanophytes extracts (without living cells) with lower costs, and to elucidate the molecular and physiological mechanisms around the plant-biofertilizer interaction, so a better understanding of its effects and how to manage them can be achieved.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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