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Bibliographic review

Use of biostimulants in chickpea cultivation

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important legumes in the world with a production of around 9 million tons. The crop is sensitive to several abiotic stresses such as: salinity, extreme temperatures and excess or deficiency of soil moisture; in addition, it can be affected by several pests and diseases. Adverse effects of the indiscriminate use of chemical products in agriculture have led to the use of various biostimulants in chickpea as a safe alternative to the use of these products, both for the supply of nutrients to plants and for the control and management of pests and diseases, as well as for the induction of tolerance to abiotic stresses. The use of plant growth-promoting bacteria (PGPB) as biofertilizers has been one of the most widely used practices in this crop, although arbuscular mycorrhizal fungi, co-inoculation of bacteria of different genera and of bacteria and fungi have also been used. In addition, the use of other biostimulants such as algae or chitosan-based products has been reported to increase grain yield and quality. The aim of this literature review is to give an updated view on biostimulant use in chickpea cultivation, with emphasis on those based on beneficial microorganisms, algal extracts and chitosan.

Key words: *Cicer arietinum*, rhizobacteria, mycorrhizae, algae, chitosan.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is considered one of the most important legumes in the world due to its high nutritional value ⁽¹⁾, being rich in proteins, carbohydrates, minerals ⁽²⁾, starch and lipids, especially unsaturated oleic and linoleic fatty acids, and not having significant amounts of cholesterol ⁽¹⁾.

It is a crop whose yield varies, to a great extent, depending on the cultivar, the soil and climatic conditions and the cultural attentions it receives in the place where it is grown ^(1,3,4). The management scheme followed for the control of pests and diseases, which can cause considerable losses in the crop, also has a great influence on this yield ⁽¹⁾.

FAO (Food and Agriculture Organization of the United Nations) seeks to promote policies and practices that support the integration of the agricultural and productive sectors and ensure the responsible management and long-term availability of natural resources ^(5,6). As projected in the 2030 Agenda for Sustainable Development; the need for sustainable agriculture is now evident ^(5,6).

The indiscriminate use of chemical products in agriculture; whether fertilizers or fungicides, has severely affected agroecosystems, contributing to the contamination of soil, water, food and even farmers ^(7,8). It has also produced nutritional imbalances in plants and has affected the ecological balance, preventing the development of soil bacteria ^(7,8). The development of pesticide-resistant pests and even the emergence of new species has also been observed ^(7,8). All this has led to the increased use of biostimulants in agriculture ^(7,9,10). A biostimulant is any substance or microorganism applied to plants with the purpose of stimulating nutritional efficiency, tolerance to abiotic stresses and crop quality, regardless of its nutrient content ⁽¹¹⁾.

According to the above definition, biostimulant categories include seaweed and plant extracts, protein hydrolysates and other N-containing compounds, humic substances, chitosan and other biopolymers, and beneficial bacteria and fungi ⁽¹¹⁾.

The main objective of this literature review is to provide an updated overview of the use of biostimulants in chickpea cultivation, mainly those based on beneficial microorganisms, seaweed extracts, and chitosan.

GENERAL INFORMATION ON CHICKPEA CULTIVATION

Around 12 million hectares of chickpeas are cultivated in the world, with a production of around 9 million tons. The main producing countries are India, Turkey, Pakistan, Iran, Mexico, Australia and Canada, while the main exporters are Turkey and Australia ⁽¹⁾.

Its yield varies greatly depending on the cultivar, soil and climatic conditions and phytotechnical management in the place where it is grown ^(1,3,4). For example, in a study carried out in Montecillo, Mexico, in two different texture soils, it was observed that urea levels affected biomass, harvest index, yield and its components ⁽⁴⁾. On the other hand, several Cuban varieties cultivated in a Ferrallitic Red soil, during 1998-1999, showed yields that ranged from 0.48 t ha⁻¹ to 2.36 t ha⁻¹ ⁽³⁾.

Chickpea *Rhizobium* can be applied to the seed, normally the symbiosis with nitrogen-fixing bacteria is sufficient to provide the necessary amounts of N, and however, the distribution of small amounts of N can be advised ⁽¹²⁾. It has also been suggested that this crop demands high levels of sulfur ⁽¹³⁾, although there are still no exhaustive studies on this subject ^(14,15).

The main pests that affect chickpea, worldwide, in the cultivation areas are: *Liriomyza cicerina* ⁽¹⁾, *Heliothis armigera* ⁽¹⁾, *Bruchus* sp. ⁽¹⁶⁾, *Plusia orichalcea* ⁽¹⁶⁾ and *Helicoverpa pagelotopoeon* ⁽¹⁷⁾, the first two being among the main pests associated with this crop in Cuba ⁽¹⁾.

In the case of diseases, the main ones are those known as chickpea rage, caused by *Ascochyta rabiei* ^(18,19) and fusariosis (mostly caused by the fungus *Fusarium oxysporum* f. sp. *ciceris*), the latter, worldwide, is one of the main limiting factors of crop yield ^(1,20). Other stresses include root rot ^(19,20) and damping-off, the main causal agents of which are *Rhizoctonia* spp. and *Botrytis* ⁽²¹⁾.

Regarding abiotic stresses, chickpea is a crop sensitive to salt stress ^(1,22-24), drought ^(22,24,25), excess moisture ⁽²⁴⁾ and low ^(22,24-26) and high temperatures ^(24,25).

The use of various biostimulants in agriculture as a safe alternative to the use of chemical products has been diversifying, either for the supply of nutrients to plants, the control and management of pests and diseases, or for the induction of tolerance to abiotic stresses ^(7,9,11). The use of some of these products for different purposes has also been increasing in chickpea cultivation.

MOST USED BIOSTIMULANTS IN CHICKPEA CULTIVATION

Among the most widely used biostimulants in chickpea cultivation are beneficial microorganisms, algae extracts and, in recent years, chitosan nanoparticles, either alone or loaded with some metals or other substances. Humic substances and Fitomas-E, among others, have also been used.

Beneficial microorganisms

Several studies have demonstrated the influence of beneficial microorganisms on nodulation, growth and yield ⁽²⁷⁻³²⁾, on grain protein content ^(27,28,33) and on biofortification ⁽³³⁾. In addition, they stimulate the N, P, K uptake, the activity of the antioxidant enzymes SOD and POD and the increase of organic acid concentrations, thereby reducing rhizosphere pH ⁽³⁴⁾. These effects are associated with the ability of these microorganisms to produce siderophores and HCN ⁽³⁵⁾, solubilize minerals such as phosphorus ^(35,36), increase root exudation ^(34,37), chelate iron ⁽³⁸⁾, fix atmospheric nitrogen ^(35,38,39), synthesize phytohormones ^(35,38,40), and inhibit infection by phytopathogens ^(27,37,41,42).

Among these microorganisms, the use of plant growth-promoting bacteria (PGPB) has become one of the most attractive options to improve the sustainability of agricultural systems in many parts of the world, due to its respect for the environment, the low cost of production and the reduced use of non-renewable resources ⁽³⁸⁾; being one of the most used practices in chickpea crop ⁽⁴³⁾.

Among these bacteria, the nitrogen fixing bacteria of the genus *Rhizobium* are the most used in this crop ^(39,40,43-47). The application of bacteria of this genus to chickpea seeds has normally been sufficient to provide the necessary amounts of N to plants; nevertheless, it is advisable to supply small amounts of this mineral ⁽⁴⁴⁾.

In a previous study, it was reported that the application of two Cuban strains of *Mesorhizobium* sp. from a suspension in water at a ratio of 1:10 (v:v) enhanced both growth (height, diameter, total plant mass and dry mass of nodules) and yield (number and pod mass and number and mass of grains per plant) ⁽³⁹⁾. Similar results were obtained in Spain, with two isolated strains of the genus *Mesorhizobium* (FCAP 26 and FCAP 04), which were able to increase the number of nodules and enhance plant development under greenhouse and field conditions, as well as to increase grain production ⁽⁴⁶⁾.

In Montecillo, Mexico State, inoculation of chickpea seeds with *Rhizobium etli* stimulated leaf area index, greenness index and grain yield ⁽⁴⁸⁾.

In addition, *Rhizobium* strains have been shown to produce volatile compounds that inhibit the growth of some soil pathogens such as *R. solani* ⁽²⁷⁾.

Other bacteria have also shown their positive effects on chickpea cultivation. Thus, it was found that the use of bacteria of *Azotobacter vinelandii* and *Burkholderia cepacia* species, in a degraded and compacted sodic lateritic soil of clayey texture from Mexico, with an organic matter content of 1.5 % and organic N 39 kg ha⁻¹ and with 50 % nitrogenous background, stimulated the growth and plant development ⁽⁴⁹⁾. In India, commercial liquid biofertilizers containing *Azotobacter* strains or phosphate solubilizers showed beneficial effects on germination and average sprout length in controlled environments and on yield under controlled and field conditions ⁽³⁶⁾.

In studies conducted for the *Fusarium oxysporum* control in chickpea, it was found that isolates of *Trichoderma viride* and *Trichoderma harzianum*, besides decreasing the incidence of *Fusarium wilt* ⁽⁵⁰⁾, improved seed germination ^(50,51), stimulated plant growth indicators such as root and sprout length ⁽⁵¹⁾ and dry mass ⁽⁵⁰⁾, as well as yield ^(50,51). On the other hand, in Argentina, when evaluating the biocontrol activity of *Trichoderma atroviride* against soil pathogens, a lower incidence of diseases during the crop cycle was found, where a higher biocontrol power was observed when used on the seed together with a biopolymer, although these results were also observed when used only on the seed and applied to the soil ⁽⁵²⁾.

Bacteria of the genus *Pseudomonas* are also widely used for growth and yield stimulation in chickpea and it has been demonstrated their effectiveness to reduce the use of chemical fertilizer ⁽⁵³⁾, besides reducing in *in vitro* tests the growth of *Rhizoctonia bataticola* and *Sclerotinias clerotiorum* and applied to seeds, they reduce in field tests the incidence of dry root rot disease and stem rot caused by these fungi ⁽⁵⁴⁾. *Pseudomonas aeruginosa* was also shown to suppress wilt and root rot caused by *Fusarium oxysporum* f. sp. *ciceris* and *Rhizoctonia solani*, respectively ⁽⁴⁰⁾. The species *Pseudomonas fluorescens* influenced vascular wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* ⁽⁵⁵⁾ and salt-tolerant species of the genus *Pseudomonas* have increased the health of chickpea subjected to salinity stress, observing that *Pseudomonas putida* RA modulated the expression of genes sensitive to salt stress ⁽⁵⁶⁾.

In another study, two P-Zn solubilizing bacterial strains (*Bacillus* sp. strain AZ17 and *Pseudomonas* sp. strain AZ5) increased grain yield, nodule number, nodule dry mass, and Zn and P uptake in two types of chickpea grown in fertilized and unfertilized soil, with better results obtained with the *Pseudomonas* sp. strain ⁽³¹⁾. It has also been reported that *Pseudomonas putida* NBRIRA and *Bacillus amyloliquefaciens* NBRISN13 strains, both alone and in consortium, were able to improve drought stress in sensitive and tolerant chickpea cultivars; obtaining a better response when strains were used in consortium ⁽⁵⁷⁾. Inoculation with two isolates of the genus *Bacillus*, from the rhizosphere of chickpea plants, promoted plant growth under greenhouse conditions and presented strong *in vitro* antagonism to *F. oxysporum*, *F. solani* and *R. solani*, in addition to the production of siderophores in CAS medium, solubilization of inorganic phosphorus and production of gibberellic acid ⁽¹⁸⁾. It has been proved that *Bacillus subtilis* influenced the vascular wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* ⁽⁵⁵⁾.

Other results have shown that isolates of *Serratia marcescens* increased crop grain yield in fertile soils in irrigated areas and nutrient-deficient soils in rainfed areas ⁽⁵⁸⁾ and strains of *Streptomyces* sp. exhibited greater number and mass of nodules, as well as greater root

and sprout mass at 30 days after sowing (DAS) and increased the number and pod mass, leaf area, leaf and stem masses at 60 DAS, and consequently, at harvest, grain yield was higher ⁽³⁸⁾.

Likewise, inoculation with a strain of *Azospirillum lipoferum* (FK1) improved the salinity tolerance of chickpea plants, expressed by significant stimulation of nutrient uptake, biomass, photosynthetic pigment synthesis, gas exchange, phenol and flavonoid content, and enzymatic and non-enzymatic antioxidant levels. In addition, inoculated plants revealed lower percentages of electrolyte efflux, H₂O₂ and MDA and exhibited high levels of expression of genes related to salt tolerance ⁽⁵⁹⁾.

On the other hand, a strain of *Aneurinibacillus migulanus* (FSZ 28) isolated from nodules of *Cicer arietinum* L. grown in soil from Fuentesauco locality in Zamora (Spain), showed the ability to inhibit the growth of different *Fusarium* species ⁽⁴⁶⁾. More recently, a plant growth-promoting rhizobacterial strain identified as *Cedecea davisae* RS3 was isolated from the rhizosphere of chickpea plants, which improved crop performance under nitrogen deficit conditions ⁽⁶⁰⁾.

Favorable results have also been found with the co-inoculation of PGPB. Thus, in greenhouse trials, it was found that co-inoculation with *Mesorhizobium* sp. FCAP 26 and *Bacillus halotolerans* FSZ 47 stimulated plant growth and development and seed production. In addition, a rotation cycle with wheat increased soil carbon and nitrogen content ⁽⁴⁶⁾.

Other results showed that co-inoculation with *Bacillus lentus*, *Pseudomonas putida* and *Trichoderma harzianum*, produced the highest yield in grains, besides, it propitiated a higher content of N, P₂O₅, K₂O, Fe and Mg in leaves and grains, nutrients that play a fundamental role in the synthesis of chlorophylls and photosynthesis ⁽⁶¹⁾. On the other hand, co-inoculation with *Rhizobium* and phosphate solubilizing bacteria stimulated dry mass accumulation, yield and grain protein content ⁽⁶²⁾.

Microorganisms based on arbuscular mycorrhizal fungi, AMF, have also been used in this crop ^(27,33,47), which stimulated crop productivity ⁽³³⁾ and improved the P, Mn, K, Cu and Fe absorption in plants ⁽⁶³⁾.

Inoculation of chickpea with arbuscular mycorrhizal fungi *Funneliformis mosseae* and *Rhizophagus irregularis*, increased plant biomass and yield and they were effective in improving the nutritional value of the grain by stimulating the concentration of proteins, Fe and Zn, these results being greater when an inoculum of local origin was used instead of a foreign one ⁽³³⁾.

It has been proven that inoculation with AMF and co-inoculation with AMF + *Rhizobium etli* increased the height, number and mass of grains per plant ⁽⁶⁴⁾, while co-inoculation

with *Rhizobium*, AMF and phosphate solubilizing bacteria significantly improved plant growth and yield indicators ⁽⁴⁵⁾.

The above results demonstrate the effectiveness of inoculation of chickpea seeds with both growth-promoting rhizobacteria (GPRB) and AMF or their combination to stimulate plant growth and development, which translates into increased yield and improved grain nutritional quality. On the other hand, plant tolerance to abiotic stresses is stimulated, as well as the growth of different pathogens that cause diseases in the chickpea crop is inhibited.

Algae-based products

The use of seaweed and seaweed products has been extended to different crops ⁽⁶⁵⁻⁶⁸⁾ and in chickpea, its use is also reported to stimulate yield and counteract the damaging effects induced by pests, diseases and abiotic stresses ⁽⁶⁹⁻⁷¹⁾.

Two applications of 1 mL L⁻¹ of seaweed extract induced significant promoting effects on growth and yield and induced favorable changes in seed quality and protein pattern profile of chickpea. In addition, it induced favorable changes in the anatomical structure of leaves and stems, mainly due to a marked increase in the thickness of bark, phloem and xylem tissues ⁽⁶⁹⁾.

Similar results show that foliar application of extracts prepared from *Kappaphycus alvarezzi* and *Gracilaria* sp. algae at 10 % significantly improved yield and its components ⁽¹³⁾.

On the other hand, inoculation with the cyanobacterium *Anabaena laxa* and co-inoculation of an *Anabaena laxa-Rhizobium* biofilm stimulated the leghemoglobin content of nodules, as well as nitrogen fixation, available nitrogen and soil microbial activity, which had a favorable effect on plant growth and grain yield, the best result being obtained with the *Anabaena laxa* inoculation ⁽²⁾.

When chickpea plants were treated with the microalgae *Chroococcus minutus* and distillery effluents, a better percentage of germination, growth and development of the plants was observed ⁽⁷²⁾. It has also been observed that the use of cyanobacteria *Nostoc commune* and *Anabaena circinalis* isolated in southeastern Iraq, increased the ability of plants to fix nitrogen, which influenced crop growth and yield, helping to a 30-50 % reduction of chemical fertilizer ⁽⁷³⁾.

In chickpea tissues treated with polysaccharide preparations of *Hypnea musciformis* (red algae), *Padina tetrastrum* (brown algae) and *Ulva lactuca* (green algae), induced phytoalexins were identified ⁽⁶⁷⁾. A biotic stress resistance inducer (k-carrageenan), obtained from *Hypnea musciformis*, was also found to induce phytoalexins in seed tissues ⁽⁷⁴⁾. In addition, the application of a solution of this polysaccharide around the seeds at the time

of sowing stimulated growth indicators, induced early flowering and produced a high content of secondary metabolites associated with disease resistance in leaves, stems and grains of plants ⁽⁷⁵⁾.

Other results show that *Spirulina* phenolic extracts have antifungal activity against *Fusarium graminearum* ^(76,77). On the other hand, extracts of *Sargassum muticum* and *Jania rubens* improved growth indicators in chickpea plants subjected to salt stress and stimulated the activities of superoxide dismutase and peroxidase enzymes. At the same time, four key amino acids, including serine, threonine, proline and aspartic acid, were identified in these extracts from their roots, which contribute to improve tolerance to salt stress ⁽⁷⁸⁾. In addition, the use of *Ascophyllum nodosum* extracts has been recommended to reduce the negative effects of drought stress on chickpea seed germination ⁽⁷¹⁾.

Products formulated with algae in chickpea not only have effects on germination stimulation, growth and yield, but also help to improve crop quality and stimulate tolerance to different abiotic stresses, in addition to having antifungal properties.

Chitosan-based products

Chitosan is a biopolymer that acts as a plant growth promoter in some crops, increases yields and protects plants against pathogens. It has a significant effect on root and stem growth and stimulates flowering and flower number. These molecules are strongly hydrophilic and attenuate the damage caused by stress in plant cells ⁽⁷⁹⁾. For these reasons it has been used by farmers as a biopesticide and biofertilizer since the 80's of the last century and for this it has been applied as a soil amendment, by foliar spraying, to fruits and seeds, both alone and in combination with other treatments to prevent the development of diseases in plants or to accelerate their innate defenses against pathogens ⁽⁸⁰⁻⁸³⁾.

In chickpea cultivation, it has been shown that, during germination, seeds treated with chitosan excreted several proteins, which have an *in vitro* inhibitory effect on the growth of the fungus *Fusarium oxysporum* f. sp. *Ciceris*, these exudates protect seeds from soil pathogens during germination ⁽⁸⁴⁾. More recently, 325 proteins and 65 metabolites associated with the chitosan-stimulated immune response to *Fusarium* were identified in this crop, which are related to the production of active oxygen species, stomatal movement, nodule development, and root architecture ⁽⁸⁵⁾.

In recent years, chitosan nanoparticles can act as growth stimulators and as antimicrobial agents against pathogenic fungi and bacteria in agriculture. In addition, they can act as nanoconductors for other existing agrochemicals ⁽⁸⁶⁾. Thus, it has been demonstrated that chitosan and chitosan-metal nanocomposites showed good antifungal activity against *Fusarium oxysporum* f. sp. *ciceris* in chickpea crop and also stimulated plant growth

compared to control plants. Chitosan-CuO and chitosan-ZnO nanocomposites were highlighted in reducing the disease caused by the pathogen ⁽⁸⁷⁾.

Also, chitosan-Ag nanoparticles showed a substantial growth-promoting effect, given by a stimulation in seed germination, length and fresh and dry mass of plants. An increase in chlorophyll content and in the activities of ascorbate peroxidase, catalase and peroxidase enzymes was found, which opens the possibility of using these nanoparticles as growth stimulators in chickpea cultivation ⁽⁸⁸⁾. A positive effect on germination, growth and induction of defensive enzymes in chickpea plants was also found when thiamine-loaded chitosan nanoparticles were used ⁽⁸⁹⁾.

All these results reveal the potential of chitosan-based products to be used as growth and yield stimulators and as bioprotectors against the attack of certain pathogens in chickpea crops.

Other biostimulants

Several results have shown that the application of certain doses of humic acids to the soil at sowing and pre-flowering stimulated growth and grain yield ⁽⁹⁰⁾, as well as protein yield of chickpea plants of the 'Çağatay' variety in Turkey ⁽⁹¹⁾. Similar results were subsequently reported for yield stimulation and yield components in plants subjected to different irrigation regimes ⁽⁹²⁾.

Foliar spraying of humic acids and naphthaleneacetic acid (NAA) stimulated plant growth by increasing indicators such as height, number of branches, leaf area, total dry mass, as well as yield components such as number of pods per plant, 100-seed mass and seed yield per hectare ⁽⁹³⁾.

On the other hand, the efficacy of humic acids in reducing the severity of wilt of chickpea plants cv. Giza 3, caused by the fungus *Fusarium oxysporum* f. sp. *ciceris*, has also been reported ⁽⁹⁴⁾.

In Cuba, Fitomas-E use, at a rate of 0.5 L ha⁻¹ on chickpea plants variety N-27, increased the number of pods and grains per plant, the mass of 100 grains and yield, under normal conditions and under drought stress ⁽²³⁾.

Treatment of chickpea seeds subjected to mild osmotic stress with ellagic acid (50 ppm), isolated and purified from *Padina boryana* Thivy, accelerated germination and seedling growth; it also stimulated total antioxidant capacity by increasing some antioxidant metabolites and enzymes ⁽⁹⁵⁾.

CONCLUSIONS

- The use of biostimulants to increase agricultural yields and for the prevention and treatment of pests and plant diseases has become widespread in different crops, and chickpea has been no exception. The most widely used have been those based on beneficial microorganisms, with emphasis on plant growth-promoting bacteria, including those of the genus *Rhizobium*, which can replace 50-100 % of nitrogen fertilizer by means of biological fixation of atmospheric nitrogen. However, the results showed that inoculation with rhizobacteria of other genera has also been beneficial.
- In recent times, co-inoculation with bacteria of different genera has been widely used, as it has proved to be an effective way to increase yields and reduce the incidence of pests and diseases in the crop. On the other hand, co-inoculation of PGPB and AMF has also been used, since it has been shown that rhizobacteria favor mycorrhizal infection of plants, which results in a greater absorption of water and nutrients and, therefore, in a stimulation of plant growth and development.
- In general, the use of these microbial biostimulants is very convenient, since it avoids soil degradation, contributes to reestablish the microbial balance and reduces environmental pollution by reducing the use of agrochemicals. On the other hand, the use of soil nutrients is increased, promoting growth, yield and reducing the harmful effect caused by different abiotic stresses. In addition, the root system is protected from infection by pathogens present in the soil by activating the plant's defensive mechanisms.
- At present, there are numerous biostimulants at the international level based on algae extracts, which have been used in various crops with beneficial results. Similarly, chitosan has been widely used in agriculture, given its characteristics not only to stimulate growth and yield, but also its ability to stimulate the defensive response of plants against the attack of certain pathogens. In chickpea cultivation, although in recent years attention has been given to the use of chitosan nanoparticles, it is necessary to further increase the use of all these biostimulants.

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