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Journal of Biomedical Engineering and Biotechnology

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Knowledge Area

The works must be unpublished and refer to topics of forest science, phytopathology, horticulture, fisheries and aquaculture, agricultural bio technology and other topics related to Biotechnology and Agricultural Sciences.

Presentation of Content

As first article we present, *Production system with two stems with low, medium and high densities in a hydroponic tomato crop*, by HERMENEGILDO-GONZÁLES, Santiago, MARTINEZ-RUIZ, Antonio, GARCÍA-MARTINEZ, Perpetua and MENDOZA-PÉREZ, Cándido, with adscription in the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) and Colegio de Postgraduados, as a second article we present, *Development and implementation of biodegradable hydrogels based on carboxymethyl cellulose for agricultural sectors*, by GRANADOS-OLVERA, Jorge Alberto, HERNANDEZ-MONTOYA, David Ian, RANGEL-RUIZ, Karelia Liliana and VARGAS-SOLANO, Zaira, with adscription in the Universidad Politécnica de Cuautitlán Izcalli and Universidad Tecnológica Fidel Velázquez, as third article we present, *Comparison of the effect of oxidant pulses on conidia under culture and free conidia*, by CASTILLO-MINJAREZ, José Miguel Angel, MARTÍNEZ-VALDEZ, Francisco Javier, CHÁVEZES-CALANTE, Luis Geovani and GÓMEZ-SÁNCHEZ, Ángel David, with adscription in the Universidad Tecnológica de Tecámac, as last article we present, *Beneficial functions of soil microbiome for sustainable agriculture*, by GUEVARA-HERNANDEZ, Eduardo, CARDONA-MARTINEZ, Clara, RODRIGUEZ-UGARTE, Maria Elena1 and ROCHA, Jorge, with adscription in the Universidad Tecnológica de Querétaro and Centro de Investigaciones Biológicas del Noroeste.

Content

Article **Journal of Biomedical Engineering and Biotechnology**
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Production system with two stems with low, medium and high densities in a hydroponic tomato crop

Sistema de producción con dos tallos para baja, mediana y alta densidades en un cultivo de jitomate hidropónico

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Abstract

In the present work, a two-stem production management system was evaluated, for low density (2.6 plants m^{-2}), medium (3.5 plants m^{-2}) and high density (4.2 plants m^{-2}), in a tomato crop (*Solanum lycopersicum* L.) hydroponic. The evaluation consisted of making comparisons of the leaf area (LA), leaf area index (LAI), dry biomass, fresh biomass, and yield. An experimental trial was established with the cultivation of tomato in the spring-summer cycle of the year 2021, in the facilities of the National Institute of Agricultural and Livestock Forestry Research (INIFAP), San Martinito Experimental Field, Puebla. The experimental units were installed in a greenhouse with overhead ventilation. The transplant of the crop was carried out on April 27, 2021, using "*tepetzil*" as a substrate and a drip irrigation system. The results showed better growth and production variables in low density, followed by medium density, and finally, high density. The highest performance was presented by the density of 4.3 plants m-2, followed by the density of 3.5 plants m-2, and finally, the density of 2.6 plants m-2.

***Solanum lycopersicum* L., planting framework, Photosynthesis**

Resumen

En el presente trabajo se evaluó un sistema de manejo producción a dos tallos, para baja densidad (2.6 plantas m^{-2}), mediana (3.5 plantas m^{-2}) y alta (4.2 plantas m^{-2}), en un cultivo de jitomate (*Solanum lycopersicum* L.) hidropónico. La evaluación consistió en realizar las comparaciones del área foliar (AF), índice de área foliar (IAF), biomasa seca, biomasa fresca, rendimiento. Se estableció un ensayo experimental con el cultivo de jitomate de ciclo de primavera-verano del año 2021, en las instalaciones del Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), Campo experimental San Martinito, Puebla. Las unidades experimentales se instalaron en un invernadero con ventilación cenital. El trasplante del cultivo se llevó a cabo el 27 de abril del 2021, utilizando "*tepetzil*" como sustrato y un sistema de riego por goteo. Los resultados mostraron mejores variables de crecimiento y producción en la densidad baja, seguida de la densidad media, por último, la densidad alta. El mayor rendimiento lo presentó la densidad de 4.3 plantas m^{-2}, seguida de la densidad 3.5 plantas m^{-2}, finalmente, la densidad 2.6 plantas m^{-2}.

***Solanum lycopersicum* L., marco de plantación, Fotosíntesis**

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Introduction

The tomato (Solanum *lycopersicum* L.) is the most important vegetable at national and international level, the crop is demanding due to its great consumption, harvested surface and economic value. It is cultivated in many regions, mainly in arid climates, and is consumed for fresh production and agro-industrial use. The production of tomato is in constant growth, due to the increase of cultivated areas and new technologies that allow higher yields. This crop is characterised by being intensive, and is grown all year round by small, medium and large producers (López, 2016).

The use of greenhouses or shade houses is a production alternative and a marketing opportunity for products grown under these systems, since they offer protection against adverse weather conditions to crops, giving them better quality and higher yields (Corella *et al*., 2013). Greenhouse cultivation has been complemented by other technologies, including hydroponics with or without substrate, in which the management of the nutrient solution and irrigation are among the most important aspects. This type of system has resulted in higher crop quality and yields, as well as efficient use of water and fertilisers (Salazar *et al*., 2014).

Tomato yield is influenced by planting density, which plays an important role in increasing or decreasing the number of plants per unit area, which is attributed to the effect on dry matter production. Therefore, it is important to assess which planting density may be the most appropriate to maximise yield.

The characterisation of production systems provides a framework within which new agricultural development strategies can be identified. Based on the above, the objective of the present study was to evaluate the two-stem management system for low, medium and high density hydroponic tomato in an intensive system, analysing the main growth and production variables, in order to obtain general deductions, both on a regional and global scale.

Materials and methods

Experimental site

The present investigation was carried out at the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), San Martinito experimental field, located at the Federal Highway Mexico-Puebla km 56.5, C.P. 74100 Santa Rita Tlahuapan, Pue., whose coordinates are: 19°20'49.9" north latitude and 98°33'57.1" west longitude and an altitude of 2538 m.

Establishment of the experimental units

The experiment was set up in a greenhouse with natural zenithal ventilation, with a N-S orientation and covered by a layer of 720-gauge plastic sheeting 12 m wide x 52 m long. A two-stem production system was established, considering each stem as a plant. The pots were arranged in six double-row beds, in a three-bowl arrangement. Saladette tomato seed (*Solanum lycopersicum* L.) cv. "*Montezuma* F1" (Harris Moran) was used during spring-summer.

Sowing was carried out on 17 February 2021, in 200-cavity polystyrene trays. Polyethylene bags of 40 x 40 cm (13 L) were filled with this substrate. Transplanting took place on 27 April, and the culture duration was up to 11 clusters per stem. The experiment was set up using a completely randomised block experimental design consisting of three planting densities as treatments: Treatment 1 (density of 2.6 plants m^2), Treatment 2 (density of 3.5 plants m^2) and Treatment 3 (density of 4.3 plants m^2). Analyses of variance for yield and leaf area were carried out using Tukey's mean comparison tests with a significance level ($\alpha = 95\%$), using the Statistical Analysis System (SAS, 2004).

Irrigation system and nutrient solution

The drip irrigation system consisted of the insertion of self-compensating drippers of 8 L h^{-1}, with adapters with four outlets, each of these with their respective tubes and stakes placed in each pot, with a semi-automated system for programming irrigation, with which 12 irrigations of one minute each were programmed. This watering time was increased according to the development and growth of the plants.

The universal solution of Steiner (1984) cited by Martínez-Ruiz et al., (2019; 2020; 2021) was applied. In the vegetative stage, N (NO_3-:11, meq L^{-1}) was decreased by 1 meq L^{-1} and K (K^+:7.5, me L^{-1}) was increased by 1 meq L^{-1} in the fruit set and fruit set stage (reproductive stage).

Leaf area of the crop

Leaf area was measured every 10 days, from the beginning to the end of the experiment. Four plants per treatment were taken at random and brought to the laboratory to measure the length and width of each leaf. Leaf area was estimated using allometric models, based on non-linear regressions, which correlate the characteristic leaf dimensions of: length, width and combinations between them (Martinez-Ruiz 2019 and 2021) and with density the leaf area index was obtained.

Biomass production

For the determination of fresh and dry biomass, the same plants whose leaf area was determined in the laboratory were used, and the fresh weight of each of the plant organs was measured: leaves + flowers, stem + rachis and fruits. Subsequently, they were placed in bags and dried in a drying oven "TECSA", at a temperature of 72 ºC, until a constant weight was obtained using an analytical balance model 1500-2M (KERN EW), the total biomass was obtained from the sum of the weights of all the organs of the plant.

Crop yield

To measure yield, eight plants per block were randomly selected, each pot was labelled and identified with coloured ribbons in each treatment, which were followed throughout the growing cycle. The tomatoes were cut as they reached maturity and the weight of each plant was recorded. At the end of the experimental trial, the weight of each of the measurements recorded for each plant during the whole crop cycle was integrated; the total weight was considered as the yield value per stem.

Results

Leaf area index (LAI)

The values obtained for LA and LAI in the three treatments are shown in Figure 1, where the evolution of the LAI is observed, which are similar in the initial stage (start of transplanting) up to 40 days after transplanting (DAT). However, after 40 DAT, the IAI increased exponentially, with values of 0.5 m^2 m^{-2} for density 2.6 plants m-2, 0.7 m^2 m^{-2} for 3.5 plants m^{-2} and 0.9 m^2 m^{-2} for 4.3 plants m^{-2}. This exponential trend was maintained, reaching 3.5 m^2 m^{-2}, 4.08 m^2 m^{-2} and 6.03 m^2 m^{-2} (for 2.6, 3.5 and 4.3 plants m^{-2}, respectively).

From 93 DAT onwards, fruit growth and development was affected, since, being in a fruiting stage, the increase in leaf area stopped until a plateau was formed (93 DAT). At 114 DAT the fruit harvest began; at this stage the LAI is affected by the fall of leaves in the senescence stage, this behaviour is also reflected in the cultivation of poblano chili under greenhouse conditions reported by Mendoza-Pérez et al. (2017). The maximum LAI values obtained were 4.35 m^2 m^{-2}, 6.04 m^2 m^{-2} and 8.71 m^2 m^{-2}, for density 2.6, 3.5 and 4.3 plants m^{-2} respectively.

The density 4.3 plants m^{-2} showed the highest value, due to a higher number of plants per unit area. This behaviour was similar to the results found by Ruelas-Islas et al. (2022), where the maximum values of leaf area index for a two-stem production system was 4.66 m^2 m^{-2}, for a density of 3 plants m^{-2} in hydroponic greenhouse cultivation.

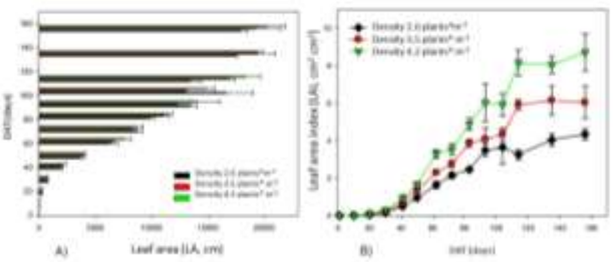

Figure 1 leaf area and leaf area index of the tomato crop in greenhouses

DDT	Phenological stage	D	AF
41	Vegetative stage	2.6	1930.7 a
		3.5	1948.0 a
		4.3	2150.1 a
62	Flowering and fruit set	2.6	6336.6 b
		3.5	6579.1 a
		4.3	7687.1 a
93	Fruit development	2.6	13419 a
		3.5	11660 a
		4.3	14017 a
114	Beginning of harvest	2.6	12564 b
		3.5	16902 a
		4.3	18972 a
156	End of harvest	2.6	16717 b
		3.5	17262 ab
		4.3	20839 a

AF=leaf area (g plant^{-1}), D=density (plant m^{-2}), Means not sharing a letter are significantly different (Tukey, $p \leq 0.05$).

Table 2 Comparison of leaf areas for three different planting densities in hydroponic tomato cultivation in greenhouses

Fresh biomass

The accumulation of fresh biomass in the different planting densities of tomato is shown in Figure 2, where the partitioning of fresh matter in the different plant organs such as leaves (Figure 2A), stems (Figure 2B) and fruits (Figure 2C) and the production of total fresh biomass (Figure 2D) are presented. According to the phenology of the crop, the accumulation and distribution of fresh matter per organ showed a similar trend throughout the crop cycle for the three densities evaluated. In general, it can be seen that, from the beginning of the growth period, the leaves, together with the stems, show an exponential trend until reaching 120 DAT, after which the fruit is the organ that significantly increases its weight due to the full fruiting stage; this organ contributed considerably to the weight values for total fresh matter (Figure 2C).

Evaluations of the behaviour of fresh matter throughout the crop cycle are also mentioned by Juárez-Maldonado et al. (2015) in which they emphasise the same trends in timing. At 156 DAT (end of harvest) there were maximum values for leaves of 823.37 g plant^{-1} for density 2.6 plants m^{-2}, 845.01 g plant^{-1} for density 3.5 plants m^{-2} and 826.70 g plant^{-1} for density 4.3 plants m^{-2}. For stems, values found were 422.21 g, 468.11 g and 420.17 g plant^{-1}, respectively.

For the fresh biomass of fruits, Figure 2C shows an exponential growth from 93 DAT, where the stage of development and maturation of the fruits begins, as Peil and Gálvez (2005) point out when mentioning that "the first phase is of vegetative growth, then the fruits begin their development, where the remaining organs of the plant continue their decelerated growth, so that the fruits constitute the main organs of greater demand that compete among themselves and with the vegetative organs for the available assimilates".

The highest weight of fresh biomass resulted for the density 2.6 plants m-2, having better development conditions, however, it is observed that at the end of the cycle the density 3.5 plants m^{-2} increases its value in leaves, stem and fruits, this is due to the slower growth, but at the end of the cycle the growth of the plants was accelerated, so it showed higher weight. This crop, having an indeterminate growth, shows a continuous production of flower clusters and fruit production. This is corroborated by the total fresh matter data for all organs (Figure 2D).

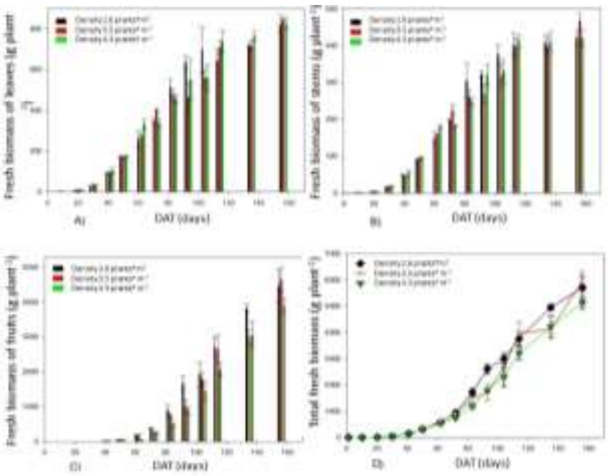

Figure 2 Fresh biomass of leaves A), stems B), fruits C) and total accumulation D) of the hydroponic tomato crop. DDT= days after transplanting

Dry biomass

Figure 3 shows the growth dynamics of leaves (Figure 3A), stems (Figure 3B) and fruits (Figure 3C), as well as the total dry matter (Figure 3D), resulting from the sum of the weights of the three organs. For the case of total dry biomass (Figure 3D), in the density 2.6 plants m^{-2}, its behaviour was of an exponential growth until 93 DAT, decelerating its development rate from those days after transplanting (fruit development), after 104 DAT it returns to its linear growth, until 120 DAT, then the development of the crop tends to stabilize, until the end of the cycle.

While the density 3.5 and 4.2 plants m^{-2} show the same exponential growth, from the beginning until reaching 114 DAT and a linear growth at 135 DAT. For the density 3.5 plants m^{-2}, an exponential increase was obtained in the course of the harvest until reaching its maximum value, while the density 4.3 plants m^{-2} maintains its linear growth. This indicates that the change in the increase in dry biomass at the harvest stage is influenced by nutrient demand, climatic conditions and planting density.

Similarly, it was found that at a density of 2.6 plants m^{-2}, 468.43 g plant^{-1} of total dry matter was accumulated, of which 24.90% was in leaves, 15.60% in stems and 59.50% in fruits. At the density 3.5 plants m^{-2}, the accumulation of total dry matter was 393.69 g plants m^{-1}, corresponding to 24.74% leaves, 17.23% stems and 58.03% fruits.

The density 4.3 plants m^{-2}, resulted in a total of 27.23% leaves, 16.96% stems and 55.81% fruits, corresponding to 27.23% leaves, 16.96% stems and 55.81% fruits, of 332.57 g plants m^{-1} of total dry matter. In this regard, Núñez *et al*. (2012) reported values of 46% for leaves and stems and 54% for fruits, slightly lower values for the densities 2.6 and 3.5 found in this research. Vargas *et al*. (2014) reinforce the results found in this study, who report that the greatest proportion of dry matter is destined to the fruits and a smaller proportion to the leaf tissues to foliar tissues.

Density m^2	Yield (kg plant $^{-1}$)		Performance (kg m^{-2})	
2.6	5.55	a	14.46	c
3.5	4.93	b	17.27	b
4.3	4.53	b	19.50	a

Means that do not share a letter are significantly different (Tukey, $p \leq 0.05$).

Table 3 Analysis of variance of yield for three planting densities of a hydroponic tomato crop in a greenhouse

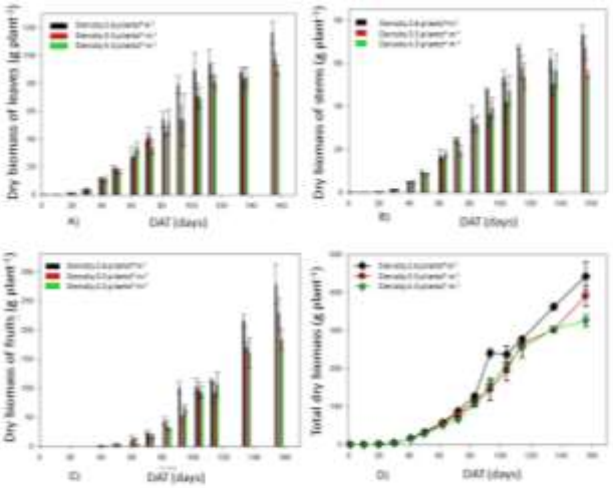

Figure 3 Dry biomass of leaves A), stems B), fruits C) and total accumulation D) of the hydroponic tomato crop. DDT = days after transplanting

Yield

During the time of the study, a total of nine fruit cuts were made and these were summed up to obtain the yield in each of the treatments. The density of 2.6 plants m^{-2}, showed a significantly higher weight per plant (Table 3), with a total of 5.55 kg plant^{-1}, followed by the density of 3.5 plants m^{-2} with a value of 4.93 kg plant^{-1} and, finally, the density of 4.3 plants m^{-2} with a value of 4.53 kg plant^{-1} and, finally, the density of 4.3 plants m^{-2} with a value of 4.53 kg plan^{-1}.

The highest yield was obtained at the high density, and the yields found were: 19.50, 17.27 and 14.46 kg m^{-2}, for densities 4.3, 3.5 and 2.6 plants m^{-2}, respectively. This is equivalent to: 144.55 t ha-1 for density 2.6 plants m^{-2}, 172.74 t ha^{-1} for density 3.5 plants m^{-2} and 195.05 t ha^{-1} for density 4.3 plants m^{-2}, finding significant differences between the three yields when expressing this variable with these units (Table 3). Mendoza-Pérez *et al*. (2018) reported close values, when increasing the number of stems, for a density of 3 plants m^{-2} in a similar hydroponic system. On the other hand, Grijalva, (2010) obtained a yield of 29.6 kg m^{-2} for a density of 3.78 plants m^{-2} worked to a single stem, grown in soil under greenhouse.

Conclusions

Under the conditions in which the present study was carried out, the crop showed higher yields at higher stocking densities, and the production of fresh matter, dry matter and leaf area index increased.

With this, it can be concluded that the density 2.6 plants m^{-2}, having higher fresh and dry biomass, yield per plant, as well as larger and higher quality fruits, was the treatment that presented better results. This was followed by density 3.5 plants m^{-2} and, finally, density 4.3 plants m^{-2}, where the plants and stems were more vigorous, but the fruits were smaller and developed more slowly.

The density 4.3 plants m^{-2} considerably increased the yield expressed in kilograms per surface area, compared to the other two treatments, and was 11.44% higher than the density 3.5 plants m^{-2} and 25.9% higher than the density 2.6 plants m^{-2}. Therefore, working this hydroponic system at different densities allows obtaining production of different quality, whose target market depends on the size of the fruit. Hydroponics continues and will continue to be a technology that allows higher yields and higher quality of harvested products.

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Development and Implementation of Biodegradable Hydrogels Based on Carboxymethyl Cellulose for Agricultural Sectors

Desarrollo e Implementación de Hidrogeles Biodegradables a Base de Carboximetilcelulosa para Sectores Agrícolas

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Abstract

This study aims to prepare superabsorbent hydrogels (PSA) from carboxymethyl cellulose (CMC), a water-soluble polymer, through crosslinking with $Al_2(SO_4)_3$, $CaCl_2$ and two comonomers. PSA evaluation included measuring gel content, swelling degree, and soluble fraction, comparing them with non-crosslinked CMC. Pitahaya growth tests in soils enriched with PSA demonstrated superior performance for CMC and $Al_2(SO_4)_3$-based PSA. Results showed a soluble fraction of 93.37%, gel fraction of 6.33%, and swelling degree of 1480.82, corresponding to water retention 15 times the weight of dehydrated PSA. The research highlights the effectiveness of CMC crosslinking for obtaining superabsorbent hydrogels with potential agricultural applications.

Hydrogels, Carboxymethyl Cellulose, Crosslinking

Resumen

En el presente trabajo se propone la preparación de hidrogeles superabsorbentes (PSA) a partir de carboximetilcelulosa (CMC), un polímero soluble en agua, mediante un proceso de entrecruzamiento con $Al_2(SO_4)_3$, $CaCl_2$ y dos comonómeros. La evaluación de los PSA se realizó mediante la medición del contenido de gel, grado de hinchamiento y fracción soluble, comparándolos con CMC no entrecruzada. Se llevaron a cabo pruebas de crecimiento de pitahayas en tierras enriquecidas con PSA, evidenciando un rendimiento superior del PSA a base de CMC y $Al_2(SO_4)_3$. Los resultados indicaron una fracción soluble del 93.37%, fracción de gel del 6.33%, y un grado de hinchamiento de 1480.82, equivalente a una retención de agua 15 veces el peso del PSA deshidratado. La investigación destaca la efectividad del entrecruzamiento de CMC para obtener hidrogeles superabsorbentes con potenciales aplicaciones agrícolas.

Hidrogeles, Carboximetilcelulosa, Entrecruzamiento

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1. Introduction

Hydrogels are three-dimensional matrices made up of polymeric networks. They have captured the interest of the scientific community since their discovery by Wichterle and Lím in 1960. Their unique ability to retain large volumes of water and their versatility position them as materials with great potential in various fields, such as food, biomaterials, agriculture, and water purification (C. Chang, L. Zhang, 2011). In this context, the present study focuses on obtaining superabsorbent hydrogels from carboxymethylcellulose (CMC) through a cross-linking process with divalent salts.

Carboxymethylcellulose, a water-soluble polymer, has proven to have significant applications in food additives, textiles, pharmaceuticals, and cosmetics. Although it has been extensively explored in various fields, its potential as a base material for obtaining superabsorbent hydrogels by cross-linking with divalent salts has been largely unexplored. The adoption of this technique offers substantial added value compared to other hydrogel formation methodologies (Bajpai et al., 2008). As noted in previous studies, CMC could respond to external stimuli, such as changes in solvent composition, temperature variations, pH fluctuations, among others (Sanna, 2013).

Unlike conventional hydrogels, superabsorbent hydrogels possess the exceptional ability to retain significantly larger volumes of water compared to their dehydrated weight. This study focuses on the preparation of CMC superabsorbent hydrogels through a cross-linking process with $Al_2(SO_4)_3$, $CaCl_2$ and two different comonomers. Evaluation of the performance of hydrogels is carried out by measuring the percentage of gel content, degree of swelling, and soluble fraction compared to non-intersected CMC. In addition, the viability of these hydrogels in agricultural applications is investigated, specifically in the growth of dragon fruit in soils enriched with superabsorbent hydrogels.

The interest in CMC-based superabsorbent hydrogels is based on their renewable origin and the potential ability to improve soil physical properties, reduce irrigation frequency, and promote healthier plant growth (Stahl et al., 2000; Weerawarna, 2009).

However, despite their advantages, the systematic exploration of the production and application of these hydrogels is still limited, which motivates the present investigation.

In this context, the aim is to optimize the production of CMC hydrogels by cross-linking with $Al_2(SO_4)_3$ and comonomers, thus exploring new perspectives in the use of these versatile materials.

2. Methodology

Materials and method

Sodium carboxymethyl cellulose (CMC) was purchased from Sigma Aldrich and was used without the need for any further purification process. All other chemical reagents used were of analytical grade. Distilled water was used throughout the experiment.

CMC Hydrogel Synthesis

The preparation of the CMC hydrogel was carried out following the methods published by (Che Nan, et al., 2019) and (Fidelia, N., et al., 2011). An 8% stock solution of CMC in distilled water was prepared (Figure 1).

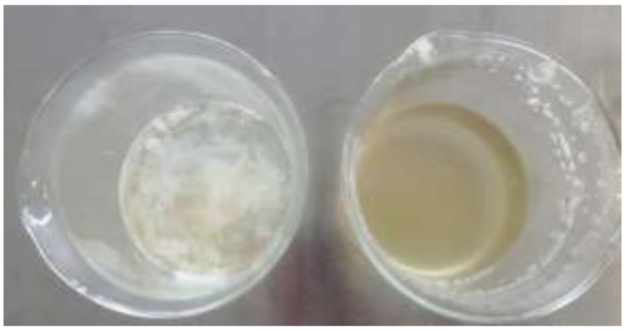

Figure 1 Preparation of 8% carboxymethylcellulose (CMC) solution in distilled water

From this solution, three different aqueous solutions were generated to investigate optimal cross-linking. These solutions contained 0.2% $Al_2(SO_4)_3$; In the second, 0.4% sodium alginate was added, and in the third, 0.2% acrylamide.

The CMC hydrogel obtained from each sample was transferred to glass containers and allowed to react at room temperature for 2 hours. Subsequently, the solution was spread on steel trays and dried at 65°C until a thin film of the dried hydrogel was formed. The film was crushed and then pulverized with a mortar.

This was repeated for each type of agent investigated, which were evaluated by determining the gel content and the degree of swelling (Figure 2).

Figure 2 Examples of the various types of hydrogels manufactured and extracted from the original solution

On the other hand, the preparation of the hydrogel in calcium chloride solution was carried out according to the method of (Chia et al., 2015), using variable concentrations of CMC and calcium. The resulting solution was allowed to react for 24 hours at room temperature, and the optimization was determined by the percentage of gel content and the degree of swelling.

The optimization of CMC hydrogels was determined by the percentage of gel content and the degree of swelling.

Determination of Soluble Fraction and Gel Fraction

The soluble fraction and gel fraction were calculated using the following formulas:

$$\text{Soluble fraction (\%)} = \frac{(Wi-Wo)}{Wi} * 100 \quad (1)$$

Gel fraction (%) = 100 – Soluble fraction
Where Wi is the initial weight of the sample and Wo is the weight of the sample after the drying process.

Determination of the Degree of Swelling

The degree of swelling was determined with the equation:

$$\text{Degree of swelling} = \frac{(Wi-Wo)}{Wo} * 100 \quad (2)$$

Where Wi is the initial weight of the sample and Wo is the weight of the sample after the drying process.

Flowerpot Experiment

Petri dishes were used to grow seeds, incorporating different polymers into the soil mixture. Pitahayas (*Selenicereus undatus*) were planted, and water was applied according to the established schedule (Table 1).

Number of days after planting	Amount of water per day (ml)
0	2
7	1
14	0.5
21	0
28	0

Table 1 Seed irrigation program

3. Results

Soluble Fraction, Gel Fraction, and Degree of Swelling

The swelling capacity of the hydrogel shows an inversely proportional relationship with the gel fraction, which means that a high percentage of gel results in a low degree of swelling due to the limited availability of spaces for the entry of free water into the hydrogel network. hydrogel (Table 2 and 3). The hydrophilicity of the carboxylic group in CMC hydrogels influences this swelling behavior (Jamingan et al., 2015). The lowest degree of swelling is observed when the gel content is highest, due to the limited space available for water. The swelling process is divided into three stages: diffusion of water molecules, relaxation of polymer chains and expansion of the polymer network (Che Nan, et al., 2019). This behavior was manifested when the CMC hydrogel was immersed in deionized water, generating osmotic pressure, and leading to the swelling of the hydrogel matrix (Abd El-salam Deghiedy, 2004).

Flowerpot Experiment

During the first two weeks, the seeds were optimally watered. Subsequently, the irrigation frequency was reduced to evaluate the response of the plants to drought conditions.

Seeds in soil without PSA enrichment ceased growth after two weeks and showed obvious signs of dehydration. In contrast, seeds in PSA-enriched soil continued to grow beyond the first two weeks, appearing noticeably healthier (Figure 3).

Figure 3 Petri dishes containing hydrogels that promoted seed development despite drought conditions

4. Annexes

SAP	$Al_2(SO_4)_3$ (%)	Alginate (gr)	Acrylamide (gr)	Soluble fraction (%)	Gel fraction (%)	Swelling degree
0	0	0	0	84.69	15.31	553.11
1	0.4	0	0	93.67	6.33	1480.82
2	0.4	0.5	0	79.19	20.81	380.48
3	0.4	1	0	83.35	16.65	500.43
4	0.4	0	0.5	92.90	7.10	1308.55
5	0.4	0	1	80.34	19.66	408.72

Table 2 Detail of the reagent concentrations for each PSA generated with $Al_2(SO_4)_3$, along with the results of the gel fraction and swelling index. All tests were carried out with a CMC concentration of 8%

SAP	$CaCl_2$ (%)	Alginate (gr)	Acrylamide (gr)	Soluble fraction (%)	Gel fraction (%)	Swelling degree
0	0	0	0	78.20	21.80	475.67
1	0.4	0	0	87.80	12.20	1190.41
2	0.4	0.5	0	73.70	26.30	300.19
3	0.4	1	0	78.01	21.99	425.37
4	0.4	0	0.5	87.75	12.25	954.26
5	0.4	0	1	75.24	24.76	324.36

Table 3 Summary of reagent concentrations in each PSA produced with $CaCl_2$, complemented by gel fraction, and swelling coefficient data. All tests were carried out with a CMC concentration of 8%

5. Conclusions

The results obtained in this study support the feasibility and promising potential of superabsorbent polymers derived from carboxymethyl cellulose (CMC) cross-linked with $Al_2(SO_4)_3$ as an ecological soil amendment. Optimization of the CMC hydrogel was achieved with a concentration of 8% CMC, 0.4% Al_2 $(SO_4)_3$, and a reaction time of 2 hours at room temperature. Application of this superabsorbent polymer to soil revealed significant improvements in water retention during periods of water stress.

The findings indicate that soil amended with this superabsorbent polymer supports healthy plant growth, especially compared to unamended soil.

This phenomenon translates into greater resistance of plants to water stress, evidencing the importance of water retention in the soil during irrigation. Furthermore, the results suggest that this approach could represent a sustainable alternative to petroleum-based superabsorbent polymers, highlighting its potential to improve water efficiency in agriculture.

For future research, it is recommended to further explore application conditions, cross-linking agents and evaluate the long-term impact of incorporating these superabsorbent polymers in various soil types and crops.

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Comparison of the effect of oxidant pulses on conidia under culture and free conidia

Comparación del efecto de pulsos oxidantes sobre conidios bajo cultivo y conidios libres

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Abstract

The objectives of sustainable development demand ecosystem-friendly agricultural practices, so the use of entomopathogenic fungi as a technology for pest control is very convenient. Oxidant pulses are an alternative method for improving the quality of conidia that could be implemented in the production chain. However, it has not been determined at what time their implementation could be more convenient, either during the cultivation stages or when the conidia have already been separated from the culture medium. Therefore, the objective of this study was to compare for the first time the effect of oxidant pulses on the quality of conidia of entomopathogenic fungi, making a simultaneous evaluation in both conditions (conidia in culture medium vs. free conidia). Using three genera of fungi of common commercial use, under the study conditions it is concluded that it is better to apply the treatment on free conidia of *Beauveria bassiana* and *Metarhizium robertsii*. On the contrary, with *Cordyceps fumosorosea*, it is better to apply the treatment on the conidia that remain in the culture medium.

Biopesticides, Sustainable Agriculture, Conidia

Resumen

Los objetivos del desarrollo sostenible demandan prácticas agrícolas amigables con los ecosistemas, por lo que el uso de hongos entomopatógenos como tecnología para el control de plagas resulta muy conveniente. Los pulsos oxidantes son una alternativa como método de mejora de la calidad de los conidios que podría ser implementado en la cadena de producción. Sin embargo, no se ha determinado en qué momento podría ser más conveniente su implementación, ya sea durante las etapas de cultivo o cuando los conidios ya han sido separados del medio de cultivo. Por lo tanto, el objetivo de este estudio fue comparar por primera vez el efecto de pulsos oxidantes sobre la calidad de conidios de hongos entomopatógenos, haciendo una evaluación simultánea en ambas condiciones (conidios en medio de cultivo vs conidios libres). Empleando tres géneros de hongos de uso comercial común, bajo las condiciones de estudio se concluye que, es mejor aplicar el tratamiento sobre los conidios libres de Beauveria bassiana y Metarhizium robertsii. Por el contrario, con Cordyceps fumosorosea, es mejor aplicar el tratamiento sobre los conidios que se mantienen en el medio de cultivo.

Bioplaguicidas, Agricultura Sostenible, Conidios

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Introduction

Achieving the sustainability vision outlined in the 2030 Agenda demands a transformative shift in how we apply new technologies, particularly in agriculture. As United Nations (UN) Secretary-General António Guterres stated, "We must transform the way we produce, consume, and waste food." Sustainable Development Goal 2 (SDG2) demands that we end hunger and achieve food security by 2030. This includes not only improved nutrition and eradicated hunger, but also fostering sustainable agricultural practices that protect ecosystems (United Nations, 2018). One promising approach to achieving these interconnected goals lies in utilizing entomopathogenic fungal conidia as biopesticides for pest control. This offers a safer and more environmentally friendly alternative to conventional chemical pesticides (Méndez-González *et al*., 2022; Miranda-Hernández *et al*., 2016).

The success of biopesticides in the field, hinges on the diverse arsenal of traits possessed by the fungal conidia. These adaptations equip them to overcome numerous environmental challenges, including insect defense mechanisms, harsh temperatures, salinity fluctuations, humidity extremes, and ultraviolet radiation. Essentially, a conidia's ability to navigate these hurdles determines its ultimate success as a biocontrol agent (Castillo-Minjarez *et al*., 2019; Miranda-Hernández *et al*., 2016).

Desirable quality conidial traits for biocontrol, such as high virulence, thermotolerance, and resistance to osmotic stress and UV-B radiation, can be influenced by the fungus's growth conditions (Rangel *et al*., 2015). Notably, this ability to adapt is particularly relevant in the context of oxidative stress, which can be induced by sublethal treatment with gaseous pulses rich in oxygen (oxidant pulses). This adaptation can offer significant benefits for conidial production. For instance, Tlecuitl-Beristain *et al*. (2010) found that exposing *Metarhizium anisopliae* to oxidant pulses with 26 % oxygen during growth resulted in a 100 % increase in conidial production without affecting germination, hydrophobicity, or infectivity against *Tenebrio molitor*. Such enhanced conidial traits hold promise for improving the efficacy and cost-effectiveness of biopesticides, leading to a more sustainable approach to pest control.

The impact of oxidant pulses on conidia varies depending on fungal species and treatment´s parameters. Studies reveal both positive and strain-specific outcomes. García-Ortiz *et al*. (2015) observed a remarkable increase in conidia production and enhanced thermotolerance in *Metarhizium anisopliae* exposed to 30 % oxygen pulses, without compromising viability, hydrophobicity, or infectivity. Conversely, Miranda-Hernández *et al*. (2014) reported contrasting responses in two *Cordyceps fumosorosea* (also known as *Isaria fumosorosea*), subjected pulses with 26 % oxygen: one strain exhibited a fivefold increase in conidia production, while the other showed a 50 % decrease. However, both strains displayed an improved infectivity against *Galleria mellonella* larvae, germination rate, and stress resistance thermal, and osmotic.

These divergent responses highlight the complex interplay between fungal biology and oxidative stress. Notably, the potential benefits of oxidant pulses extend beyond the growth phase. Notably, Castillo-Minjarez *et al.* (2019) demonstrated the benefits of oxidant pulses even for harvested conidia (free conidia), treating them with 26 % oxygen significantly increased osmotic stress resistance in *Metarhizium robertsii*, *Beauveria bassiana*, and one *Cordyceps javanica* strain. Additionally, germination and thermotolerance improved in *M. robertsii* and *B. bassiana.*

Driven by the escalating demand for high-quality, readily available conidia in mass production for biocontrol, oxidant pulses emerge as a promising tool for enhancing their efficacy. However, a crucial question lingers: The optimum time to apply the oxidant pulses for best results. The integration of oxidant pulses during the stages of fungal culture, while within the shelter of the culture medium, or application to the harvested conidia, already free of their nutrient source, are two options to be evaluated. Each approach presents distinct feasibility challenges and cost considerations. Cultivating under oxidative stress could demand stringent sterility measures, potentially inflating production expenses. Conversely, treating free conidia might be less costly but could potentially compromise conidial stability.

Navigating the optimal timing for oxidant pulses application requires discerning which stage yields the most potent effect.

This study embarked on a mission to compare the impact of oxidant pulses on both cultivated and free conidia of three prominent entomopathogenic fungi (*Metarhizium*, *Beauveria*, and *Cordyceps*). The focus was on assessing if observed positive effects, such as enhanced thermotolerance and stress resistance, remained comparable across both application stages. This knowledge will unlock ways for future cost-benefit analyses and pave the way for the optimal integration of oxidant pulses into large-scale conidia production, ultimately enhancing the efficacy and affordability of sustainable pest control strategies.

Methodology

1. Biological material

The microorganisms used were *Cordyceps fumosorosea* ARSEF3302 obtained from the Agricultural Research Service Culture Collection of Entomopathogenic Fungi (ARSEF), Ithaca, New York; *Metarhizium robertsii* Xoch8.1 and *Beauveria bassiana* Tac1.1, obtained from the Centro Nacional de Referencia de Control Biológico, Colima, Mexico. The strains were deposited in the ENCBIPN Culture Collection WDCM449 with identification numbers ENCB-MG-79 (*C. fumosorosea*), ENCB-MG-81 (*M. robertsii*) and ENCB-MG-82 (*B. bassiana*).

2. Initial propagation

Fungi of each species studied were grown at 27 ± 2 °C in 250 mL Erlenmeyer flasks (Pyrex) containing the surface medium here called APA, composed of 33.3 g/L oat flour (Sol Campestre®, Productos del Campo, Mexico), 15 g/L bacteriological agar (Bioxon, Mexico) and 10 g/L meat peptone (Bioxon, Mexico) (Tlecuitl-Beristain *et al.*, 2010).

3. Quantification and extraction of conidia on culture medium

After 8 to 9 days of incubation, the conidia in culture medium were extracted with 0.05 % Tween 80 (Hycel). The conidia were counted in a Neubauer chamber (Marienfeld, Lauda-Königshofen, Germany) and optical microscope (Boeco, Germany). From the conidial extracts with Tween 80, conidial suspensions of 1 x 10^8 con/mL were prepared, from which inocula were taken to perform the different experiments.

Under sterile conditions, 100 µl of the 1 x 10^8 con/ml suspensions were taken and inoculated into 120 mL serological bottles (Distbrand, Mexico) containing APA medium (Miranda-Hernández *et al.*, 2014).

4. Conidiation status

It was proposed the introduction of a new parameter called Conidiation Index (*Ic*), which easily indicates how the production of conidia in the culture medium varies over time, with respect to an initial inoculum.

The parameter is calculated with the following equation:

$$Ic = \frac{C}{Ci} \tag{1}$$

Where:
C is the observed production of conidia in each time.
Ci is the concentration of the initial inoculum.
The units of both terms are conidia per square centimeter (con/cm^2).

The study employed 100 µL of 1 x 10^8 con/mL suspensions as inoculum in serological bottles containing APA medium (section 3). This corresponds to an initial concentration of 5.76 x 10^5 conidia per square centimeter of agar surface (con/cm^2), equivalent to an *Ic* = 1. Applying equation (1), a measured concentration of 1.80 x 10^6 con/cm^2 at a specific culture time would result in:

$$Ic = \frac{C}{Ci} = \frac{1.80 \text{ x } 10^6}{5.76 \text{ x } 10^5} = 3.12$$

This would indicate that the production of conidia increased 3.12 times with respect to the initial amount of conidia in the culture.

The effect of the oxidant pulses on conidia production was determined by comparing the Ic obtained under the different atmospheric conditions studied.

5. Oxidant pulses

An oxidant pulse consisted of inserting a needle into the rubber stopper that functioned as a gas outlet and a second needle was placed, which in turn was connected to the gas tank with the oxygen mixture corresponding to 26 %; once both needles were placed.

The gas was allowed to pass for 1 min at a flow rate of 20 cm^3/s; subsequently, the needles were removed and the bottles were kept at room temperature. During the time each experiment lasted, the pulse was reapplied every 24 h (Castillo-Minjarez *et al*., 2019).

6. Assessing the effects of oxidant pulses on the quality of free conidia and conidia in culture medium

This experiment was carried out to observe the effect of oxidant pulses on the quality of conidia under culture and to compare with the effect on free conidia. The same methodologies were applied to all the fungi studied.

6.1 Harvesting conidia in culture medium and free conidia

A batch of serological bottles containing 10 mL of APA medium was made and inoculated with 100 µl of a 1 x 10^8 con/mL suspension and incubated at 27 ± 2 °C. Once cultures were at an advanced stage of conidiation (*Ic* close to 100), bottles were taken to exchange the cotton plug for the rubber stopper and start the pulse. Simultaneously, bottles were taken to scrape the conidia gently with a sterile spatula and a standardized amount of these was placed in sterile serological bottles, without culture medium, some bottles were kept with cotton plugs (control units) and others with rubber stopper to apply the oxidant pulses.

In all cases, the evaluation of the quality characteristics was made 24 h after starting the oxidant pulse and 96 h later (in the latter case, the pulses were applied every 24 h).

6.2 Viability

Under sterile conditions, 1 x 10^4 con/mL suspensions were prepared, 30 µL (300 conidia approximately) were taken and inoculated in Petri dishes with ADS (Bioxon, Mexico), incubated at 27 ± 2 °C for 60 h and the colony forming units (CFU) present were counted, determining the percentage of viability (Miranda-Hernández *et al*., 2014).

6.3 Heat resistance

The same suspensions (1 x 10^4 con/mL) used for viability were placed at 40 °C for 1 h in a thermomixer (Eppendorf, Germany).

After the time elapsed, the viability of conidia was determined as previously explained (Castillo-Minjarez *et al*., 2019).

6.4 Osmotic stress resistance

The viability of conidia from different atmospheric conditions and from different species was evaluated, as previously described, in this case the 30 µL of 1 x 10^4 con/mL suspensions were inoculated in Petri dishes with ADS (Bioxon, Mexico) and NaCl (Meyer, Mexico) 0.5 M (Castillo-Minjarez *et al*., 2019).

7 Statistical analyses

Each experiment was made by triplicate. Student's *t* test (α= 0.05 %) from the "Data Analysis" tool of Microsoft Excel (2010) was used.

Results

1. Effect of treatment on conidiation

Figures 1, 2 and 3 show the conidiation indexes (*Ic*) in the control cultures (normal atmosphere of 21 % oxygen) and with the oxidant pulses (26 % oxygen). It can be observed that in all cases a high conidiation state (close to or greater than 100) was present. In addition, a positive effect was observed on the treatment on production of conidia of the *Cordyceps fumosorosea* ARSEF3302 and *Metarhizium robertsii* Xoch8.1.

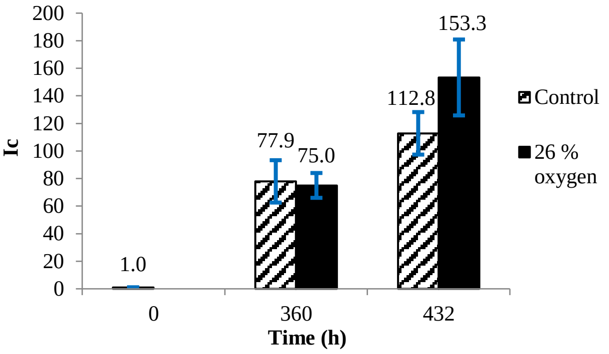


Figure 1 Effect of the oxidizing pulse on the production of conidia of the entomopathogenic fungus *Beauveria bassiana* Tac1.1
Own Source

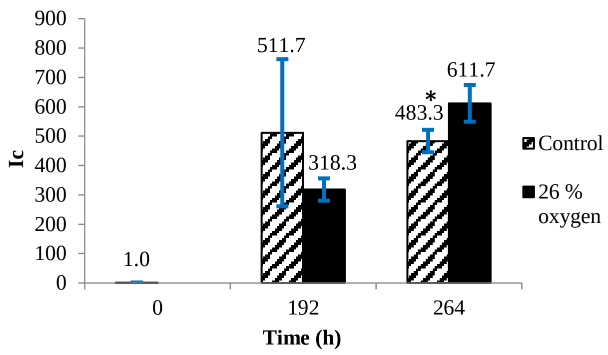


Figure 2 Effect of oxidative pulse on conidial production of the entomopathogenic fungus *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

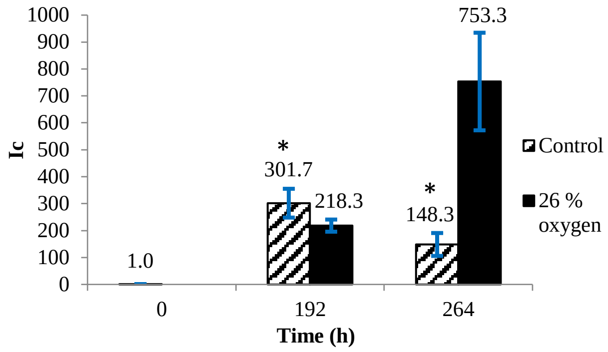


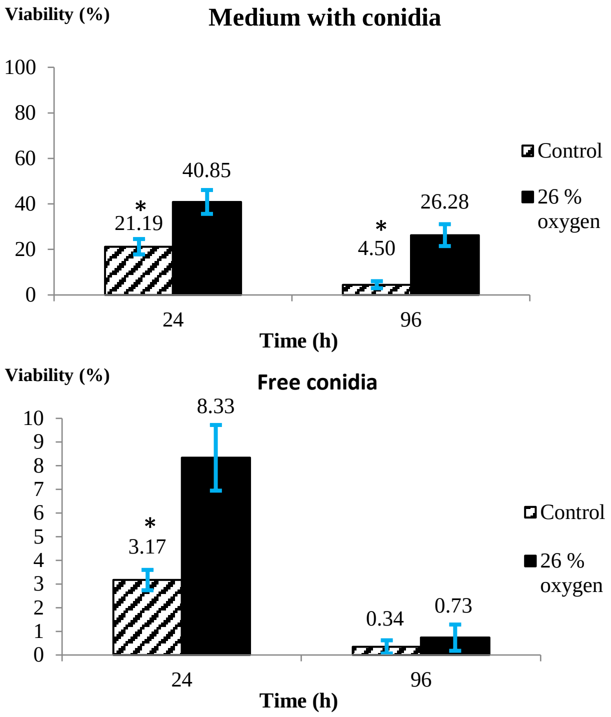
Figure 3 Effect of oxidative pulse on conidial production of the entomopathogenic fungus *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2. Evaluation of the effect of oxidative pulse on the quality of free conidia and conidia in culture medium

The following section presents the effects of the treatment on the evaluated quality characteristics, both in the cultured conidia and the free conidia.

2.1 Viability

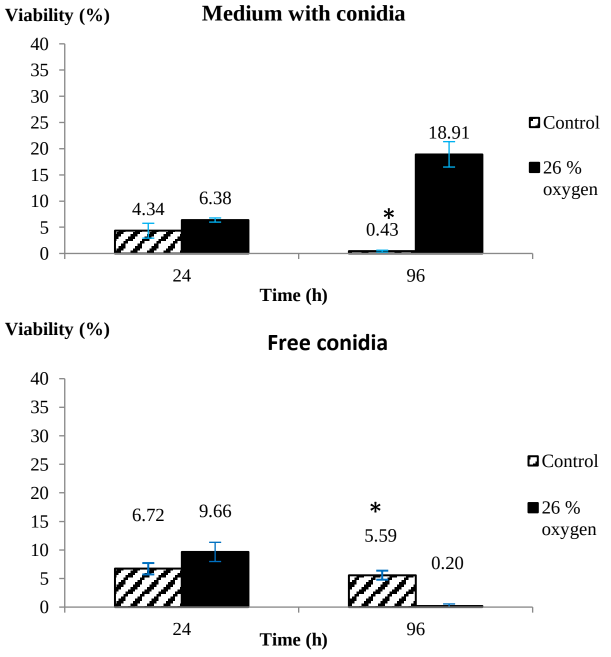
In the case of *Beauveria bassiana* Tac1.1 (Figure 4), it is observed that the viability naturally decreased in conidia that were separated from the culture medium (free conidia) compared to those that remained in it. Regarding the effect of the treatment, it was observed that oxidant pulse improved viability in conidia that remained in the culture medium, and despite the drop in viability of free conidia, there was also an improvement in this characteristic when the first pulse was applied (24 h).


Medium with conidia

Free conidia

Figure 4 Effect of treatment on viability of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 5), viability was very similar between free conidia and those maintained in the culture medium. However, this fungus presented a very low viability. Regarding the treatment effect, it was observed that the oxidant pulses improved viability in the conidia that remained in the culture medium; nevertheless, there was the opposite effect in the free conidia.


Medium with conidia

Free conidia

Figure 5 Effect of treatment on viability of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 6), it was observed that viability was very similar between free conidia and those kept in culture medium. Regarding the treatment effect, it was observed that the first oxidant pulse (24 h) improved viability in the conidia that remained in the culture medium. In contrast, free conidia showed no change.

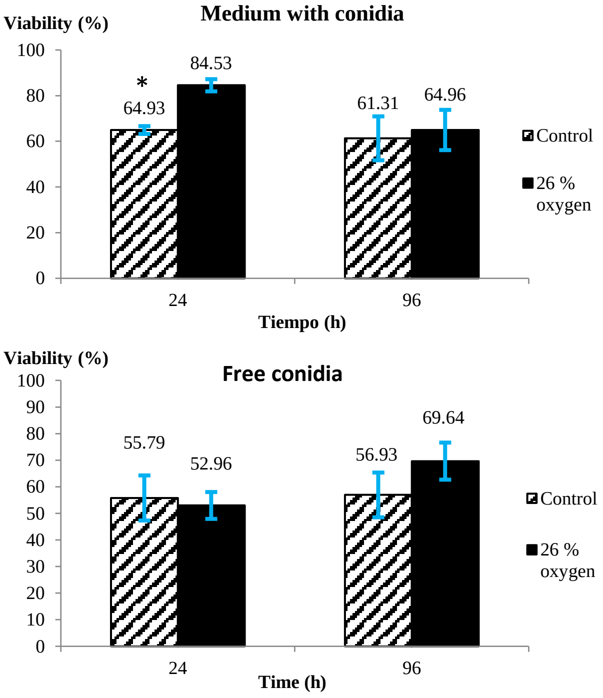
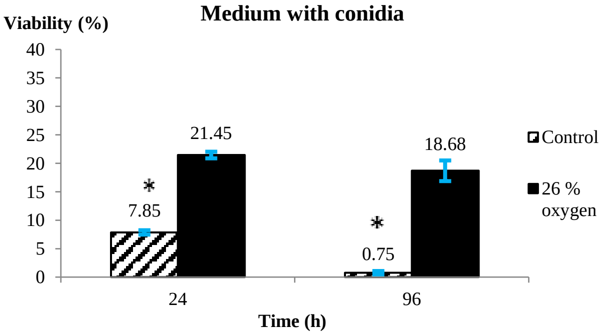


Figure 6 Effect of treatment on viability of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2.2 Heat resistance

In the case of *Beauveria bassiana* Tac1.1 (Figure 7), it was observed that the oxidant pulses improved the high temperature resistance in the conidia that remained in the culture medium and despite the drop in viability of the free conidia; there was also an improvement in the high temperature resistance when the first pulse (24 h) was applied.



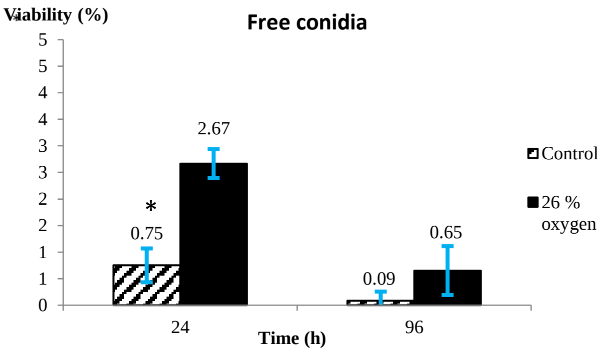


Figure 7 Effect of treatment on high temperature resistance of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 8), it was observed that the oxidant pulses improved the resistance to high temperature in the conidia that remained in the culture medium. In free conidia there was also an improvement in this characteristic when the first pulse was applied (24 h).

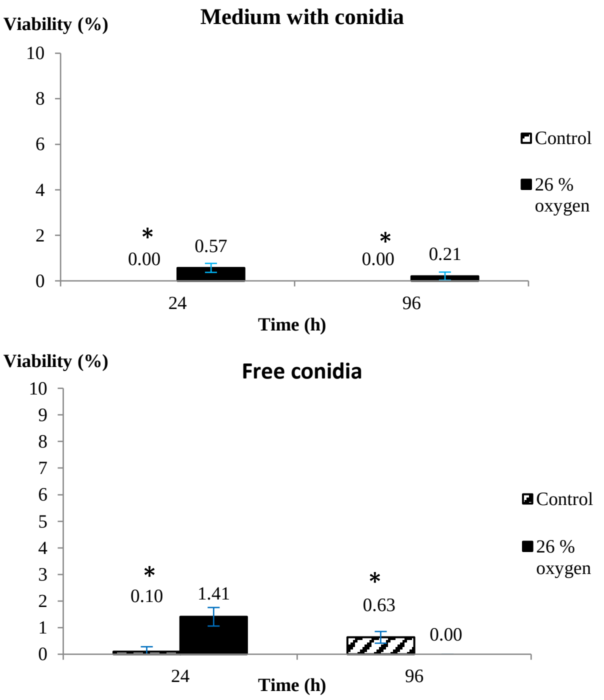


Figure 8 Effect of treatment on high temperature resistance of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 9), it was observed that the first oxidative pulse (24 h) improved the resistance to high temperature in the conidia that remained in the culture medium. Differently, in free conidia this characteristic was improved.

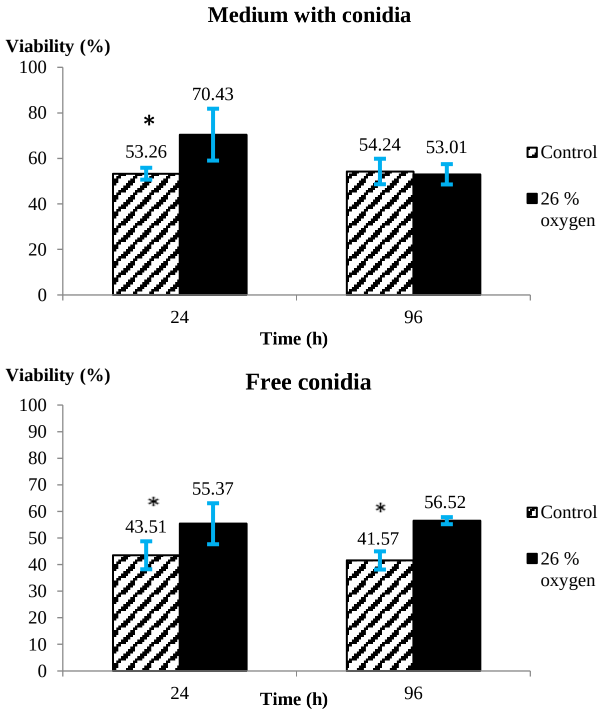


Figure 9 Effect of treatment on high temperature resistance of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2.3 Osmotic stress resistance

In the case of Beauveria bassiana Tac1.1 (Figure 10), it was observed that oxidant pulses enhanced resistance to osmotic stress in conidia remaining in the culture medium and in free conidia.

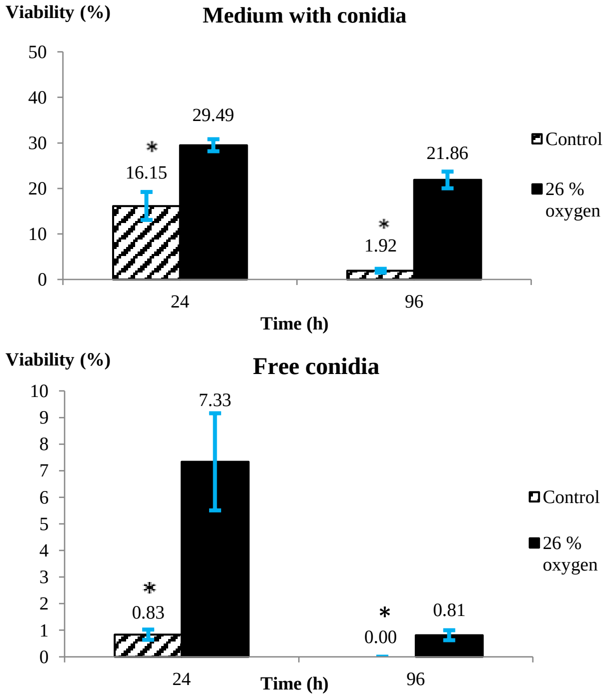


Figure 10 Effect of treatment on osmotic stress resistance of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 11), it was observed that the oxidative pulses improved the resistance to osmotic stress in the conidia that remained in the culture medium. Differently, the characteristic was not modified in free conidia upon application of the first pulse (24 h), however it was affected with constant treatment.

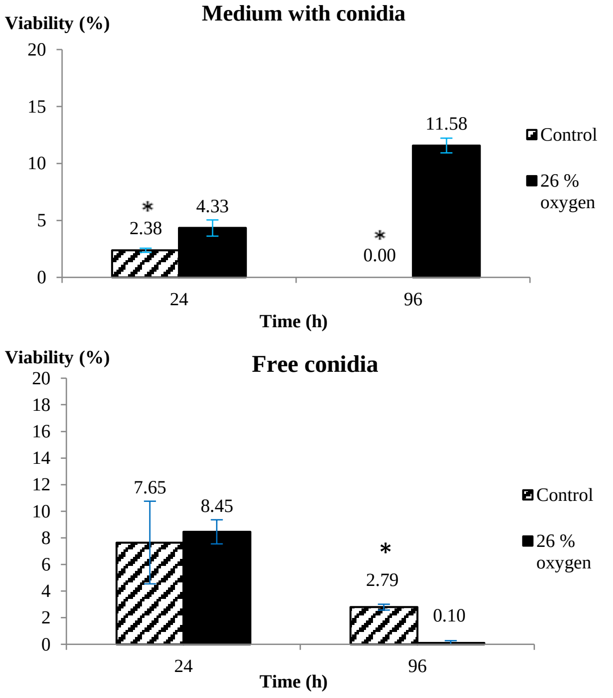
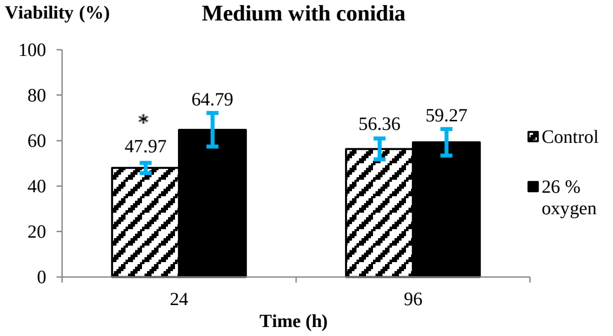


Figure 11 Effect of treatment on osmotic stress resistance of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 12), it was observed that the first oxidant pulse (24 h) improved the resistance to osmotic stress in the conidia that remained in the culture medium. With free conidia, this characteristic was improved.



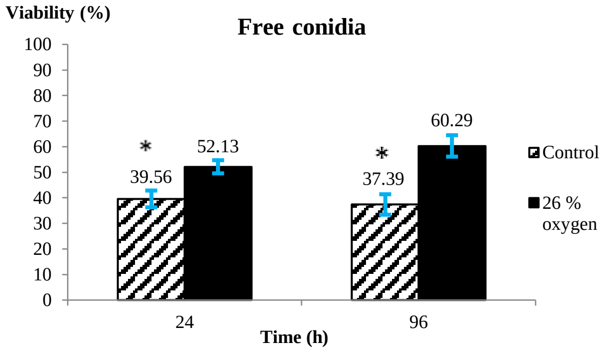


Figure 12 Effect of treatment on osmotic stress resistance of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

3. Discussion

Interestingly, it was observed that a single oxidant pulse was sufficient to improve the viability (2.1 section results) of the conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1 fungi in the culture medium; however, with *Cordyceps fumosorosea ARSEF3302* the positive effect was also present, but with more pulses. On the other hand, in the free conidia only *Beauveria bassiana* Tac5.1 showed an improvement of 160 % concerning the control, which was also greater than the improvement of 90 %, which was presented with the conidia in culture medium.

In the case of high heat resistance (2.2 section results), again only one oxidant pulse was sufficient to improve this characteristic in the conidia of the three fungi studied, both in the free state and on culture medium. With free conidia of *Beauveria bassiana* Tac5.1, there was a greater improvement (2.6-fold) compared to conidia in culture medium (1.7-fold). In the case of *Cordyceps fumosorosea* ARSEF3302, due to the low viability, the numerical treatment to quantify the changes presents much uncertainty, however, qualitatively it can be seen that the treatment favors the characteristic in the conidia with medium. In the case of free conidia it was possible to determine a 13-fold improvement, concerning the control. The conidia of *Metarhizium robertsii* Xoch8.1 showed a general improvement of approximately 30 %.

In the case of osmotic stress resistance (2.3 section results), an oxidant pulse caused a positive effect on conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1, both free and in culture medium.

With free conidia of *Beauveria bassiana* Tac5.1, there was a greater improvement (780 %) compared to conidia on culture medium (80 %). *Metarhizium robertsii* Xoch8.1 conidia, maintained the overall improvement of approximately 30 %, including a 60 % improvement when more oxidant pulses were applied to the free conidia. In the case of *Cordyceps fumosorosea* ARSEF3302, an improvement of 80 % was determined with the conidia with culture medium.

These results indicate conidia of the entomopathogenic fungi studied have the ability to respond to the sublethal treatment used in this research. In addition, they can increase their resistance to more than one adverse condition, which is congruent with what has been observed in other research (Miranda-Hernández et al., 2016; Rangel, 2011). It is possible that oxidative pulses lead to the activation of defense mechanisms of the conidia of the fungi studied, for example, Castillo-Minjarez *et al.* (2019) reported the activation of the glutathione system as a non-enzymatic defense mechanism against oxidative conditions. In turn, Garcia-Ortiz *et al.* (2018), studying the proteome of conidia from oxidant atmospheres of *Metarhizium lepidiotae* CP-OAX, found proteins involved in the activation of general stress response mechanisms. However, further molecular studies are needed to understand the response mechanisms, especially of free conidia.

Acknowledgment

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Conclusions

This is the first time that the effect of oxidant pulses on the quality of free conidia and conidia in culture medium has been studied simultaneously on entomopathogenic fungi from the same production lot. To determine in which condition there would be the best effect, it is concluded that it is better to apply the treatment on free conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1. On the contrary, in the case of *Cordyceps fumosorosea* ARSEF3302, it is better to apply the treatment to conidia on culture medium.

These results may be of interest in the case of seeking to improve the quality traits of the strains studied in their commercial production. However, in order to apply the treatment to other strains, it would be necessary to study the effect, since, as was observed in this work, the response to the oxidant pulse will depend on the genus or species of entomopathogenic fungus to be used.

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Beneficial Functions of Soil Microbiome for Sustainable Agriculture

Funciones Benéficas del Microbioma del Suelo para la Agricultura Sustentable

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Abstract

Plant-associated microbes are key factors for the health of crops; in this sense, microbial inoculates represent a promising strategy for maintaining productivity in modern agricultural production systems, while sustainably adapting to adverse climatic factors, reducing pollution and decreasing use of toxic substances for the environment. Genomic and metagenomics tools have allowed a better understanding of how intensive agriculture has negative effects on the diversity and function of the plant microbiome, and in turn, how the management of microbial communities can mitigate some negative effects of crop modernization. Here we review the beneficial roles of plant-associated bacteria, and the symbiotic interactions between the microbiome and various economically important crops; we also discuss how this knowledge can provide strategies towards a more sustainable agricultural production.

Soil microbiome, Sustentaintable agriculture, Functional microorganisms

Resumen

Los microorganismos asociados a las plantas son factores clave para la salud de los cultivos; en este sentido, los inoculantes microbianos representan una estrategia prometedora para mantener la productividad en los sistemas de producción agrícola modernos, adaptándose de manera sostenible a factores climáticos adversos, reduciendo la contaminación y disminuyendo el uso de sustancias tóxicas para el medio ambiente. Las herramientas genómicas y de metagenómica han permitido una mejor comprensión de cómo la agricultura intensiva tiene efectos negativos en la diversidad y función del microbioma de las plantas, y, a su vez, cómo el manejo de las comunidades microbianas puede mitigar algunos efectos negativos de la modernización de los cultivos. En este trabajo, revisamos los roles benéficos de las bacterias asociadas a las plantas y las interacciones simbióticas entre el microbioma y diversos cultivos de importancia económica; también discutimos cómo este conocimiento puede proporcionar estrategias para lograr una producción agrícola más sustentable.

Microbioma del suelo, Sustentabilidad agícola, Microorganismos funcionales

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Introduction

Modern agricultural production systems are focused on growing high-yielding plant varieties in monoculture systems, or sometimes, simple rotations. These require a large amount of toxic agrochemicals such as pesticides and fertilizers, and they use highly industrialized, intensive soil management. These practices are not sustainable since they cause soil eutrophication, ecosystems pollution, high water consumption, deforestation, greenhouse gas emissions and loss of biodiversity (Wagg *et al*., 2019). Under this production systems, soil and water are exploited excessively. In second half of the XX century, this production model was considered ecologically adequate, but factors such as climate change and water scarcity have become more prominent.

Therefore, agricultural production has focused on developing more sustainable systems. (Baveye *et al*., 2018). New production strategies have been implemented such as precision agriculture, that is controlled by sophisticated technologies such as IoT (Internet of Things), deep and machine Learning, AI, among others (Coulibaly *et al*., 2022; Akhter *et al*., 2022; Saranya *et al*., 2023). These systems also incorporate genetically modified organisms (Woźniak-Gientka et al., 2023; Tyczewska *et al*., 2023; Mohammadi *et al*., 2023) and development of new resistant hybrid varieties (Duarte-Aké *et al*., 2023; Paudel *et al*., 2023; Mourtala *et al*., 2023; Zhang *et al*., 2023). However, these methods need advanced technologies and sophisticated infrastructure, which are not accessible in many countries. Likewise, some countries have strict regulations on the use of GMOs.

A more accesible and sustainable alternative is the management of the soil microbiome. The soil microbiome represents 25% of the total biodiversity of the planet. The functioning of the ecosystem and soil productivity is highly dependent in the microbial communities since they control biogeochemical cycles, especially the carbon cycle. In recent decades, a large number of articles have been published demonstrating the beneficial effects of PGPB (Plant Growth Promoting Bacteria) on the health and development of plants, and in crop productivity, using various cultivation methods such as green houses, pots, or in open field.

It has been shown that PGPB facilitate the nutrient flow, increase the production of hormones, volatile or regulatory compounds in important crop plants such as Corn, (Jochum *et al*., 2019; Bomfim *et al*., 2020; Song *et al*., 2023), Wheat, (Safdarian *et al*., 2020; da Silva *et al*., 2022; Almutairi *et al*., 2023; Karimzad *et al*., 2023), Rice, (Xiao *et al*., 2020; Cavite *et al*., 2021; Pan *et al*., 2023), Sunflowers, (Furnkranz *et al*., 2009; Rojas-Tapia *et al*., 2012), Legumes (Chiappero *et al*., 2019; de la Osa *et al*., 2021; Sapre *et al*., 2022) and Arabidopsis, (Chen *et al*., 2022; do Amaral *et al*., 2023). PGPB also facilitates the absorption capacity of toxic substances such as heavy metals, and mediate the resistance of crops to adverse abiotic factors such as high salinity in soils or drought, among others (Li *et al*., 2017; Bhise *et al*., 2019; Becze *et al*., 2021; Tirry *et al*., 2021; Chatterjee & Niinemets, 2022; Sapre *et al*., 2022; Wu *et al*., 2023; Yue *et al*., 2023; Cardoso *et al*., 2023).

Throughout history, agricultural production systems have been in constant adaptation (Anderson *et al*., 2020); in present times, the main challenge is to maintain performance in food production but using more sustainable practices (Malhi *et al*., 2021). In this sense, it is essential to incorporate technological advances but also to consider the contribution of the microbiome in agricultural production. This work reviews some of the most notable functions of the microbiome that can be used in more sustainable agricultural production systems.

Microbial diversity contributes functional structure in the soil

The formation of a functional structure involves several biological processes in which bacteria and fungi generate organic substances such as mucilage, hyphae, polysaccharides, proteins, lipids, etc. These compounds can interact with biotic factors such as carbonates, metal oxides and other components present in the soils, all of which stimulate the nutrients flow between soil and plants (Yang *et al*., 2020).

In recent years, this interaction has been studied with growing interest in the rhizosphere and rhizoplane. This process depends on various factors such as the amount of hair roots, the physical structure of the surrounding soil and mucilage secreted by beneficial bacteria or by roots such as auxin (Xu *et al*., 2021). As a result, more stable organo-mineral aggregates are generated.

This structure can be formed when conditions of moderate drought occurs, since it has not been observed in flooded soils. Likewise, this structure favors tolerance to drought and acidity of the plants, improves the accumulation of phosphorus, contains a higher nitrogen content than bulk soil.

Microbial diversity also benefits from this structure, since alpha diversity increases in the endosphere and it can improve the assembly of bacterial communities in crops such as rice (Xu *et al*., 2021; Yudina *et al*., 2023). These interactions are favored when exopolysaccharide-producing microorganisms are present such as *Bacillus* and *Azozpirillum.*

These bacteria improve both soil aggregation and the water status of the leaves when plants grow in drought conditions because volatile organic compounds such as esters are released. Ethers, aldehydes, naphthyl derivatives, ketones, alkalis, and benzene derivatives facilitate interactions between microbes and induce the secretion of root exudates in rice, maize, and cotton (Aslam *et al*., 2022).

Microbes improve nutrient flow from the soil to the plant

The flow of energy and nutrients in agroecosystems is crucial because it is directly related to environmental issues such as global warming, soil eutrophication, and the generation of pollutants (Bhattacharyya *et al*., 2022). The soil microbiome mediates biogeochemical cycles that regulate these processes. For example, in the regulation of terrestrial carbon flow, some autotrophic bacteria along with plants can absorb atmospheric CO_2 (Mukhtar *et al*., 2023).

In the soil, the contribution of CO_2 emissions is 9-fold higher than emissions from anthropogenic causes and is mainly due to the decomposition of organic matter and autotrophic respiration (Dutta & Dutta, 2016; Jansson *et al*., 2020). On the other hand, some methanotrophic bacteria consume part of the CH_4 generated by the decomposition of organic matter, reducing the harmful effects of this gas on the environment, as this bacteria consume up to 60% of the CH_4 produced on the planet (Leng *et al*., 2015; Dutta & Dutta, 2016).

Therefore, the balance between the generation of these greenhouse gases by the soil and the capacity of bacteria to assimilate them can be leveraged to mitigate the environmental impact caused by agricultural production.

In the nitrogen cycle, soil bacteria also play a prominent role since the amount of N_2O generated by the soil can be up to 65% of the total. For this, the processes of nitrification and denitrification are crucial for regulating this cycle. Nitrogen fixation is mainly carried out by diazotrophic bacteria and archaea (Kuypers *et al*., 2018). Various studies have supplemented fertilizers and biofertilizers with bacteria in intensive corn and wheat crops, revealing differences in the expression of genes related to nitrogen fixation such as nifH (encoding nitrogenase reductase), amoA (encoding ammonia monooxygenase), nirK and nirS (encoding nitrite reductase), and nosZ (encoding nitrous oxide reductase) (Grzyb *et al*., 2021).

This supplementation improves soil denitrification and enhances plant growth. Additionally, it increases the abundance of PGPB associated with the decomposition of organic matter, such as Chloroflexi and Bacteroidetes, reducing nitrogen losses in the soil by 54% (Chen *et al*., 2019).

On the other hand, atmospheric ammonia (NH_3) emissions related to the use of agrochemicals can be significantly drecreased (by 44%) when using organic fertilizers. If these fertilizers are inoculated with *B. subtilis*, the reduction can reach up to 71%. This reduction is mainly addressed by considering the composition of the soil microbiota, where some genera are associated with processes such as ammonization, ammoxidation, and nitrosation (Di Benedetto *et al*., 2017; Sun *et al*., 2020). Regarding bacterial communities, differences were also found in relation to the type of fertilizer used, with a higher richness and evenness index for organic fertilizer with and without inoculation compared to compound fertilizer. Importantly, some plant growth-related bacterial genera such as *Bacillus*, *Nitrospira*, and *Rhodoplanes* were enriched when using organic fertilizer inoculated with *B. subtilis* (Sun et al., 2020).

Microbes contribute to plant growth

Plant growth promoting bacteria (PGPB) is a very important group in the soil microbiome. Plants can recruit different phyla of bacteria from the soil, such as *Actinobacteria*, Bacteroidetes, Firmicutes, and Proteobacteria, belonging to various genera like *Achromobacter, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Exiguobacterium, Flavobacterium, Gluconacetobacter, Herbaspirillum, Methylobacterium, Paenibacillus, Pseudomonas, Rhizobium, Serratia, and Staphylococcus*, as well as fungi like *Trichoderma, Laccaria,* and *Piriformospora* that promote plant growth (Kour *et al*., 2019). This interactions are facilitated by root exudates such as sugars, organic acids, amino acids, phenolic compounds, saponins, among other substances. In turn, bacteria can regulate and stimulate plant growth by producing phytohormones such as auxins, gibberellins, abscisic acid, ethylene, cytokinins, and also participate in nutrient exchange with the soil.

Hence these interactions modulate the plant response to stress induced by nutrient limitation, drought, flooding, extreme temperatures, high soil salinity, and contamination (Zuluaga et al., 2021; Chai *et al*., 2022). For example, in the wheat domestication process, these metabolites promote plant biodiversity, improve survival, provide protection against pathogens, and enhance the complexity of soil bacterial consortia (Yue *et al*., 2023).

These benefits are also observed in fungi such as *Piriformospora indica*, which promotes growth and stress resistance to drought in corn. There is a significant increase in the expression of genes related to 17 metabolic processes, including signal perception, stress response, redox regulation, transport, and distribution of proteins in different cellular organelles. Processes related to the production of phytohormones such as abscisic acid, cytokinins, ethylene, gibberellins, jasmonic acid, and brassinosteroids are particularly noteworthy. These hormones play crucial roles in the response to drought in different plant species (Zhang *et al*., 2018).

Inoculations with strains of the *Bacillus* genus also have a positive effect on various crops such as *Triticum aestivum* L., *Bromopsis inermis* L., *Pisum sativ*um L., and *Zea mays* L., *Arabidopsis*, and soybean crops, showed increased plant resistance to drought, stimulation in growth, reduction of oxidative stress, improvement in the quantity of fresh shoots and roots, chlorophyll content in leaves, increased production of biologically active metabolites, and enhanced response to auxin (Khan *et al*., 2020; García-Cárdenas *et al*., 2023; Kuramshina & Khairrullin, 2023).

Microbes mediate control of pests and pathogens

Microbes can stimulate defense mechanisms in plants by competing with other microorganisms for space or nutrients, and also inducing systemic resistance in plants through the production of antibiotics, lytic enzymes, or volatile compounds. These mechanisms are primarily regulated by the production of phytohormones, which are generated by the stimulation of secondary metabolites produced by microorganisms in the soil (Rolfe *et al*., 2019).

From a genetic perspective, in microbial communities, some secondary metabolites exuded in the roots of crops modulate the composition of bacterial and fungal communities associated with the rhizosphere, promoting plant growth, defense, and increased resistance to plant pathogens such as bacteria, viruses, protozoa, insects, and herbivores (Hu *et al*., 2018; Vannier *et al*., 2019). These effects are significant because plants can alter the composition of soil bacteria by secreting bioactive molecules into the rhizosphere, such as sugars, amino acids, carboxylic acids, as well as various secondary metabolites.

These molecules have diverse effects on the plant, serving as signaling molecules, attractants, stimulants, inhibitors, or repellents. Consequently, the accumulation of pathogens in the soil can suppress plant growth, while the accumulation of beneficial microbes, such as nitrogen-fixing bacteria or mycorrhizal fungi, can enhance plant development (Hu *et al*., 2018).

One of these molecules is benzoxazinoids, which trigger changes in the root-associated microbiome structure, leading to an increase in jasmonic acid and salicylic acid signaling pathways in the leaves. This, in turn, activates jasmonic acid-dependent defenses that suppress the growth of pathogens in plants (Yuan *et al*., 2019). Therefore, it is likely that microbes and their metabolites are transmitted and influence the beneficial bacterial assembly for the next plant generation (Hu *et al*., 2018; Yuan *et al*., 2019).

Microbes aid in pollutant degradation

Plants can recruit microorganisms in the root system for the degradation of toxic contaminants such as chemicals, pharmaceuticals, heavy metals, and nanoparticles that are introduced into the soil through pesticides, organic fertilizers, contaminated irrigation water, and microplastics (Rolli *et al*., 2021). Many of these phytoremediation processes actually take place in the rhizosphere, where the plant creates a microhabitat in the surrounding areas of the roots. In this microhabitat, oxygen, metabolites, and exudates are produced, facilitating the degradation of derivatives of aromatic compounds, polycyclic aromatics, long-chain hydrocarbons, and microplastics (Simmer & Schnoor, 2022).

Regarding soils contaminated with heavy metals, it was observed that *Bacillus cereus* strains enhance the growth capacity and phytoextraction of heavy metals by *Brassica nigra* (Aktar *et al*., 2021). On the other hand, Rizvi *et al*. (2019) analyzed wheat varieties contaminated with Ni (100 µmol mL-1) and Pb (200 µmol mL-1) grown in a culture media. These metals induced structural alterations in roots and leaves, along with a decrease in the amount of proline and malondialdehyde. However, when the seeds were inoculated with *B. subtilis*, the activity of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione S reductase increased.

This caused a lower accumulation of metals in plant tissues (roots, seeds, and shoots), allowing the plants to better survive even in soils contaminated with heavy metals.

In a different study, the growth of corn in soils contaminated with multiple heavy metals such as Pb, Ni, Zn, Cu, and Cd was analyzed, under abiotic stress caused by increasing the crop temperature to 45 ºC, and the effect of growth-promoting bacteria on corn development was examined. For this purpose, a strain of *B. cereus* was isolated and identified from soils contaminated with heavy metals, and corn seeds were inoculated. The results showed a significant increase in biomass, chlorophyll, carotenoids, and protein content, as well as enhanced antioxidant enzyme activity, compared to the non-inoculated controls (Bruno *et al*., 2021).

Conclusions

Sustainable progress of agricultural practices needs to take into account the role that microorganisms play in crop adaptation to various adverse environmental factors, enhancing crop resistance to pests and diseases, increasing plantation biodiversity, and their importance in nutrient flow between soil and crops. Parallel to technological and scientific advances in the development of precision agricultural production processes, it is evident that agricultural production is still highly dependent on natural factors. This is reflected in a significant increase in the number of organic farmers in recent years.

The benefits of some Plant Growth-Promoting Bacteria (PGPB) are still being studied and are not fully understood, and genomic and metagenomic tools are being explored to understand the function and assembly of synthetic communities more deeply to enhance these benefits. This calls for the integration of precision agricultural systems with the beneficial effects of bacteria, which can enhance the mitigation of adverse environmental impacts related to agricultural production, making it sustainable while maintaining competitive yields.

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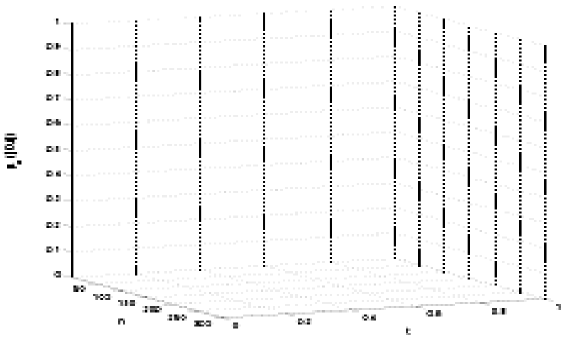

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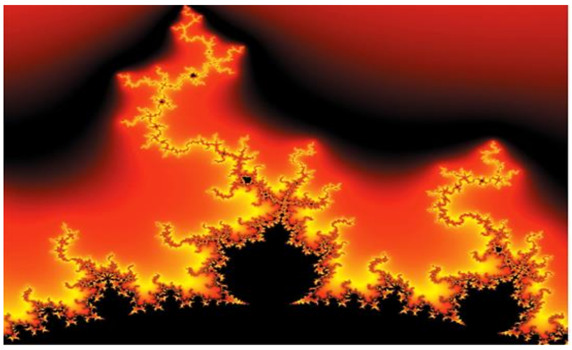

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