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Use of barley straw as a support for the production of conidiospores of *Trichoderma harzianum*

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ABSTRACT

In this work was to evaluate the conidiospores production of *Trichoderma harzianum* using barley straw as substrate. Four growth conditions were used; washed and unwashed barley straw and washed and unwashed barley straw supplemented with mineral salts. The highest spore production was observed when washed barley straw supplemented with mineral salts with 1.56×10^{10} conidiospores/gram of dry matter (gdm) at 216 h of cultivation was used. The effect of substrate moisture on spore production was studied, three initial moisture levels of the substrate were tested and it was observed that a humidity of 80 % of the substrate improves the production of conidiospores reaching a concentration of 2.35×10^{10} conidiospores/gdm at 136 h. Finally, conidiospores viability was evaluated for 12 months by keeping them on the conidia and substrate, and viability of 71 % of the conidiospores was observed, so this maintenance method is an excellent means of conserving the conidiospores viability.

1. Introduction

The natural ability of *Trichoderma* sp. to produce a large number of metabolites as well as its ability to grow on organic and lignocellulosic residues has generated great interest during the last six decades since this fungus has been used for the biological control of pests, enzyme production and fermentable sugars production, so it can be considered as a good candidate for implement new biotechnological processes or optimize existing ones [1,2]. The use of *Trichoderma* sp. for biological control of pests is complimented for its ability to stimulate the defense mechanisms of plants against phytopathogens as well as to favor the growth of soil microbial communities that promote the growth of plants that increase the yields of various commercial crops [3,4]. This capacity of *T. harzianum* is attributed to the fact that this fungus is capable of producing a large number of elicitors and enzymes and that thanks to its action, plant growth is favored.

There are at least 50 formulations based on *Trichoderma* sp. and that are used in different countries, these formulations are applied to protect and improve the yields of vegetables, ornamental plants and fruit trees [5]. These fungi are among the microorganisms most resistant to natural changes, synthetic chemicals, and toxins, as they are able to rapidly degrade some of them, so they also play an important role in the bioremediation of contaminated soil [6–8].

Currently, rice and wheat are the substrates that are mostly used for the production of spores, however, the high cost of the raw material [9], the low spore yield, and the difficulties for the quantification of the biocontrol activity limits the commercialization of formulations of conidioespores of *Trichoderma* sp. at higher scales [10], it is therefore important to have a raw material and low-cost procedures that provide the essential nutrients for the growth and sporulation of *Trichoderma* sp. [11].

An alternative for the cultivation of *Trichoderma* sp. is the use of lignocellulosic residues which are composed of lignin, cellulose, and hemicellulose, the latter biopolymers represent an excellent source of carbon for the growth of filamentous fungi. It has been described that *T. harzianum* has the capacity to secrete a wide range of enzymes that allow it to grow on lignocellulosic substrates such as barley straw [12,13]. In addition to the carbon source that represents the cellulosic material, straw contains other minor

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components that are not part of the cell wall, such as extractable compounds in water and solvents and ash, which together represent a good source of micronutrients necessary for the growth of various microorganisms [14].

The barley (*Hordeum vulgare*) belongs to the Gramineae family, cataloged according to statistics of the FAO (United Nations Organization for Food and Agriculture) as the second most important crop within the coarse grains, with a world production of around 177 million tons in 2008. Specifically, a global production of barley straw of 51.3 million tons is estimated [15], so that sufficient raw material is available to carry out processes of this type. In this work, the effect of washing and not washing barley straw as well as the presence and absence of salts during the conidiation of *T. harzianum* was evaluated. The effect of the initial moisture of the substrate on the sporulation process was also evaluated.

2. Materials and methods

2.1. Microorganism

The *Trichoderma harzianum* strain was provided by the Agro-biotechnology Laboratory of the Polytechnic University of Pachuca (UPP). The strain was cultivated on potato dextrose agar (PDA) and incubated for 7 days at 28 °C and kept at 4 °C until its use. The strain was reseeded on PDA plates every 30 days.

2.2. Substrate preparation

The barley straw used in this work was obtained from barley crops in the Zempoala region, Hidalgo, Mexico (19° 57′ 28.5″ N; 98° 41′ 18.90″ W), from the 2017 cycle, the raw material was stored in a cool, dry place and avoiding contact with sunlight. The straw was crushed using a knife mill and sieved by selecting the particles with a size of 0.84–1.67 mm. The straw was divided into two fractions; one was subjected to washing with cold water for 30 min and dried in an oven at 60 °C for 24 h. The other fraction did not receive any type of treatment. Both fractions were stored in dark plastic bags at room temperature.

2.3. Cultivation conditions and conidiospores production

The solid-state fermentation was carried out using barley straw washed with cold water and unwashed straw previously dried in an oven at 60 °C for 48 h. In both cases, the straw was crushed using a hammer mill and sieved to a particle size of 0.8 mm, then 5 g of barley straw were added in 250 mL Erlenmeyer flasks. The humidity of the straw was adjusted to the percentages tested (70, 75 and 80 %) with a solution composed of 1×10^7 conidiospores/gdm supplemented with a solution of salts and water. The fermentations were carried out for 10 days at 28 °C, samples were taken every 24 h in triplicate. To study the effect of the presence of salts on the conidiospores production the mineral medium described by Pontecorvo [16] was used without the addition of glucose.

2.4. Chemical characterization of barley straw

2.4.1. Removable material in organic solvents

The TAPPI standard T-204 om-88 was used by means of a Soxhlet system and hexane as solvent [17]. For the analysis, 10 g of each sample was used.

2.4.2. Material removable in water

The TAPPI standard T-207 om-93 was used with the residue resulting from solvent extraction [18].

2.4.3. Ash content

It was determined in accordance with the TAPPI standard T-211 om-93, for which 1 g of sample was used [19].

2.4.4. Lignin insoluble in acid

From 1 g of sample, the percentage of lignin present by weight difference was determined according to the TAPPI standard T-222 om-88 [20].

2.4.5. Holocellulose content

2 g of the sample was added 100 mL of 1.5 % sodium chlorite and glacial acetic acid. The reaction was carried out at 75 °C for 5 h. Subsequently, the mixture was filtered in a Gooch crucible, washed with acetone and dried at 105 °C for 1 h. The percentage of holocellulose was determined by the weight difference between the initial and the final.

2.4.6. C/N ratio determination

The barley straw was dried in an oven for 72 h at 60 °C, subsequently, later, the straw was ground and dried again for 24 h at 60 °C. The straw was transferred to capsules for their respective analysis. An elemental analyzer (PerkinElmer, 2400 series II) was used and the C/N ratio was calculated from the carbon and nitrogen concentration estimated by the equipment.

2.5. Measurement of enzymatic activity and conidiospores quantification

The extracts obtained during the solid fermentation were analyzed to know the number of conidiospores, xylanolytic and cellulolytic activity, as well as the amount of total extracellular protein.

2.5.1. Harvest and conidiospores quantification

The harvest of conidiospores was carried out by adding 50 mL of Tween 20 sterile (0.05 %) to each flask, stirring at room temperature for 15 min. The obtained suspension was placed in a 50 mL plastic tube and kept at 4 °C for no more than 1 month. The conidiospore count was performed using a Neubauer chamber.

2.5.2. Cellulolytic and xilanolytic activity

The sugars released due to the hydrolytic activity during the growth of the fungus on the barley straw were quantified using the modified DNS technique. Carboxymethylcellulose and birch xylan were used, both at 0.1 % to determine the cellulolytic and xylanolytic activity respectively, both substrates were dissolved in acetates buffer at 0.1 M, pH 6. Glucose and xylose were used to obtain the calibration curves. One activity unit (AU) was defined as the amount of enzyme needed to release one μmol of reducing sugar per minute per mL.

2.5.3. Protein quantification

The method described by Bradford [21] was used and bovine serum albumin was used as the standard for obtaining the calibration curve.

2.5.4. Qualitative determination of laccase activity

To determine the laccase activity on the plate, the PDA medium supplemented with 0.01 % ABTS was used. The plates were incubated at 28 °C for 2–3 days until the appearance of a green halo indicative of laccase activity.

2.6. Conidiospores viability determination

Erlenmeyer flasks of 50 mL containing 5 g of barley straw inoculated with 1×10^6 conidiospores gdm^{-1} (gram of dry matter)

were prepared. After 216 h of culture, these flasks were sealed and placed in a dark place and each month the conidiospores were harvested and counting and measuring their viability. To measure the viability of the spores with respect to time, dilutions were made from the spore solution to a dilution of 100 spores/mL, which was seeded on PDA plates, incubated at 28 °C for 5 days. The number of germinated conidiospores was observed with the help of a stereoscopic microscope. The counts were made in triplicate.

2.7. Statistical analysis

A complete factorial design 2^2 was used, the combinations were: barley straw without washing with water; unwashed barley straw with salts; straw washed with water and straw washed with salts. The results were subjected to an analysis of variance and the means were compared with Duncan and Tukey's analysis with $\alpha = 0.05$, using the statistical software SPSS version 15.0.

3. Results

3.1. Barley straw composition

The chemical characterization of the barley straw was carried out according to the TAPPI standards. The components measured in the barley straw were: extractables in solvents, extractable in water, holocellulose, ash, and lignin soluble in acid and water. The results obtained from the characterization are shown in Table 1.

3.2. Cellulolytic and xylanolytic enzymes production

The effect of the barley straw washed and the addition of salts on the production of enzymes and conidiospores was studied. The washing eliminates a large number of soluble compounds, forcing the fungus to use the hemicellulose and cellulose present in the substrate as the only source of carbon. During the washing, minerals that may be necessary for the growth of microorganisms are also eliminated, to study the effect it produces the washing and the addition of salts were assayed different growth conditions: washed and unwashed barley straw, and washed and unwashed barley but supplemented with mineral salts. During the four treatments, the production of the hydrolytic enzymes involved in the degradation of holocellulose from barley straw by *T. harzianum* was monitored. The highest cellulolytic activity was detected in the treatment of unwashed straw added with salts maintaining similar levels of enzymatic activity from 48 h to 192 h (around 10 AU/mL), later in the treatment of washed straw added with salts the highest cellulolytic activities were detected at 144 h and 192 h, no significant differences were observed with the treatment of washed straw + salts. In the case of washed and unwashed straw without the addition of salts, no significant differences were observed (Fig. 1A) between both treatments, the highest enzymatic activity was reached at 96 h of culture for both cases with 5 AU/mL. Regarding the xilanolytic activity, the largest activities were again detected in the unwashed straw and washed with addition of salts; however, the statistical analysis indicates significant differences in the four treatments, the enzymatic activities are maintained in a range of 25–35 AU/gdm for the treatments of unwashed straw, unwashed straw + salts and washed straw + salts being the straw treatment washed the most different of the four (Fig. 1B).

Figs.1A and B show the results obtained from the enzymatic kinetics, it is observed that after 24 h of growth, the enzymatic activity increases, which may be an indication that the fungus uses the available substrates first and subsequently begins the production of hydrolytic enzymes responsible for the hydrolysis of holocellulose. It was also observed that *T. harzianum* did not present problems of growth on the substrate because it was able to produce laccase when performing a qualitative test for the production of the laccase enzyme, it was observed that when growing *T. harzianum* in PDA medium supplemented with ABTS, a deep green halo was generated around the colony, indicative of the production of the extracellular laccase due to the oxidation of ABTS by enzymatic. The ability of the *Trichoderma* genus to produce laccase catalytic activity has already been reported by other authors. This enzyme, within all the functions that have been attributed to it, can participate in the degradation of lignocellulosic residues because it is capable of destabilizing the structure of lignin, facilitating its degradation.

Table 1
Chemical composition of barley straw.

Fraction	Content (%)
Ash	10.34 ± 0.12
Extractables in water	11.2 ± 0.43
Extractables in solvents	3.5 ± 0.37
Holocellulose	56.3 ± 1.45
Lignin soluble in acid	18.2 ± 0.5
Lignin soluble in water	1 ± 0.3
Initial moisture	5.6

3.3. Conidiospores production

As a next stage, the effect of the four treatments on the conidiospores production of *T. harzianum* was studied, it was observed that in the treatment of washed straw + salts at 216 h of culture, 1.56×10^{10} conidiospores/gdm were produced, followed by the treatment of unwashed barley straw without addition of salts with 9.5×10^9 conidiospores/gdm at 192 h of culture. Where the lowest number of conidiospores occurred was in the treatment of barley straw washed without the addition of salts with a maximum production of 4×10^9 conidiospores/gdm at 216 h of culture (Fig. 2). The pH had little impact on the sporulation process since it remained constant throughout the growth of the fungus.

3.4. Initial moisture effect on the conidiospores production

The results obtained in the present work showed that at higher humidity, improves sporulation, three levels of initial humidity of the substrate were tested, 70, 75 and 80 %, it was observed that at an initial humidity of 80 % the highest sporulation was detected with 2.35×10^{10} conidiospores/gdm at 136 h of incubation, at a humidity of 75 %, 2.25×10^{10} conidiospores/gdm were obtained at 128 h of incubation and with a humidity of 70 %, the maximum value of sporulation was 1.12×10^{10} conidiospores/gdm at 112 h of culture (Fig. 3). Adequate humidity is necessary to maintain all the metabolic processes of the fungus, in addition, the presence of water facilitates the diffusion of hydrolytic enzymes through the lignocellulosic substrate improving the growth of the fungus, therefore, it was observed that at higher humidity, there is more growth so the sporulation process is higher. Beyond 80 % humidity, due to the type of substrate, there is a risk of bacterial contamination due to the low water absorption capacity of barley straw.

3.5. Conidiospores viability

In the present work, conidiospores viability kinetics was carried out for 12 months to estimate how the germination of the spores is affected over time without any treatment, conserving them only on the conidia and substrate. The results obtained showed a loss of viability of 29 % during the 12 months of the study (Fig. 4).

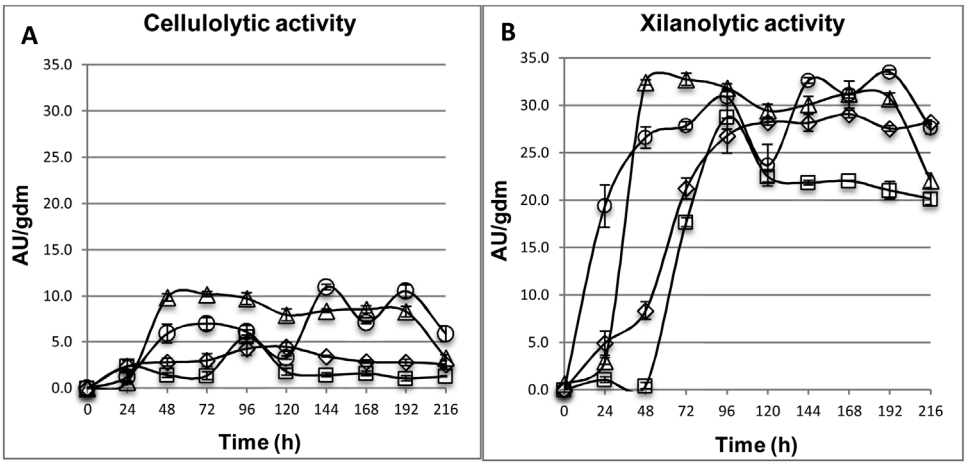

Fig. 1. Enzymatic activities of *T. harzianum* during growth on barley straw. □ = washed straw; ◇ = Unwashed straw; △= Unwashed straw + salts; ○ = Washed straw + salts.

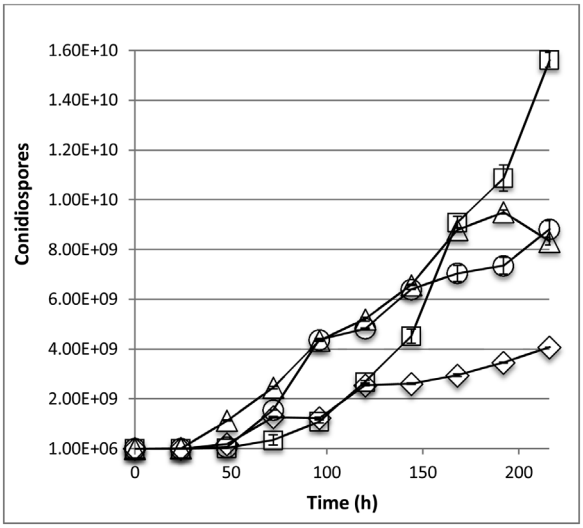

Fig. 2. Conidiospores production by *T. harzianum* in the four treatments. □ = Washed straw + salts; ◇ = Washed straw without salts; △= Unwashed straw without salts; ○ = Unwashed straw + salts.

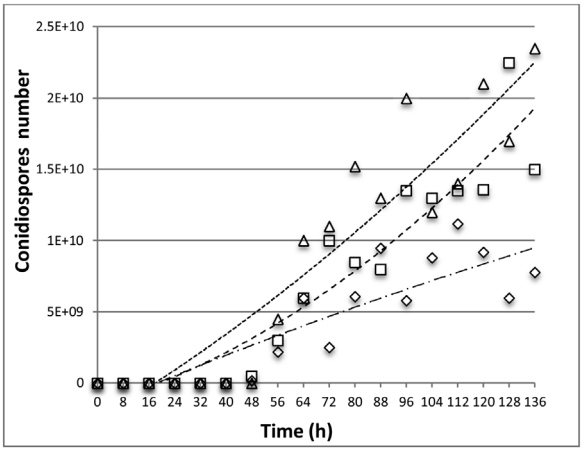

Fig. 3. Effect of the initial moisture of the substrate on the conidiospores production of *T. harzianum* grown on washed barley straw added with salts. ◇ = initial moisture of 70 %; □ = Initial moisture of 75 %; △= Initial moisture of 80 %.-·-·- = Initial moisture of 70 %; ——— = Initial moisture of 75 %; ···· = Initial moisture of 80 %.

4. Discussion

The results obtained from the characterization of barley straw are similar to those reported by other authors, the composition of barley straw is influenced by various factors that affect its chemical composition, as well as by the environmental conditions in which cultivate. This variation is clearly observed when compared with Contreras-López et al. [22], they conducted a study in which the composition of barley straw cultivated in different locations in the state of Hidalgo, Mexico was analyzed, observing differences in the concentrations of the analyzed fractions according to the variety of the plant and parts of the same. They studied barley straws from five different geographical zones of the state of Hidalgo, Mexico, and observed that the cultivation zones had a strong impact on the chemical composition of the barley straws studied, finding variations of 39.6–46.3 % for the carbohydrate content, from 4.56 to 7.23 % for the ash content and from 5.88 to 9.69 % for the moisture content. If these results are compared with results obtained from the analysis of barley straws in other latitudes, the presence of the main components of barley straw will vary even more, for example, Jin et al. [23] detected 71.9 % holocellulose and 15.8 % of lignin for barley straw cultivated in China, which makes it evident that there is no trend in the composition of this raw material. The washing of barley straw allowed the elimination of compounds such as starches, carbohydrates, inorganic compounds, gums, tannins and coloring matter that could be contained

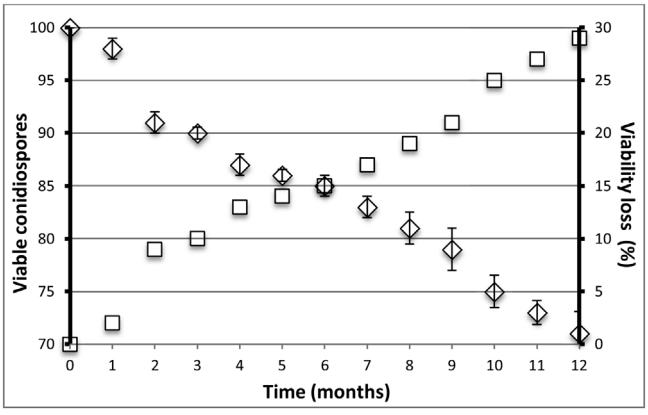

Fig. 4. Viable conidiospores of *T. harzianum* during 12 months, stored at room temperature on barley straw.

in barley straw [18]. These compounds are important at the beginning of the growth of microorganisms since they are the most available and are, in principle, those that are first consumed by microorganisms that colonize the lignocellulosic substrates, it is likely that this type of compounds is consumed by the fungus in the early stages of its growth. Ball et al. [24] found that aqueous extracts of barley straw reached a concentration of 19 % (w/w) composed mainly of lignin (8 %) and holocellulose (70 %), on the other hand, Rosgaard et al. [25], pretreated barley straw found a concentration of extractables in water of 24 % composed by monosaccharides such as glucose, xylose, and arabinose mainly, it is clear then that the aqueous extracts favor the extraction of carbohydrates while the extracts with solvents favor the extraction of compounds such as fatty acids, sterols, waxes, esters and triglycerides [26]. In the results obtained in this work, it was observed that the extractable compounds, both in water and in solvents, represent almost 15 % of straw weight, a value very close to those reported by other authors. It is important to know the composition of the extractable compounds of the lignocellulosic residues since they can be used as a substrate at the beginning of the microbial growth and subsequently favor the processes of degradation of the raw material, in our case, of the barley straw.

Regarding to lignocellulolytic enzymes production, the production of this type of enzymes using lignocellulosic residues has been well studied, Deschamps et al. [27] used a mixture of wheat straw and bran for the cellulases production of *T. harzianum*, they found up to 11 international units of cellulolytic activity, Syuan et al. [28], reported cellulolytic and xilanolytic activities of 12.7 AU/gdm and 100 AU/gdm, respectively, when *T. harzianum* grew on rice straw pretreated with acid, and depending on the acid used during the pretreatment, this has an effect on the release of hydroximethylfurans (HMF) that directly affect enzymatic activity in addition, the authors supplemented the substrate by adding peptone as a nitrogen source to rice straw. Lee et al. [29] studied the cellulolytic activity of *T. harzianum* grown on various agricultural residues including barley straw, to which different nitrogen sources were added, finding that barley straw was the best substrate for the production of cellulases complemented with soy peptone. Iqbal et al. [30] studied the production of *Trichoderma viride* cellulases grown on wheat straw pretreated with HCl found a cellulolytic activity of 398 U/mL. López-Ramírez et al. [31] found a maximum production of xylanases with 109 AU/gdm and for cellulases of 9.11 AU/gdm using *T. harzianum* and wood sawdust as substrate; they used salts supplemented with glucose as a carbon source and peptone and urea as nitrogen source. Silva et al. [32] studied the cellulases and xylanases production using sugar cane bagasse, orange peel, wheat bran, rice, and bagasse as substrates, finding that the highest production of xylanases was obtained with bagasse pretreated with NaOH with 93 U/mL and the cellulase, in orange peel supplemented with sucrose with 0.5 U/mL. Grujić et al. [33] used the lignocellulosic residue resulting from the cultivation of edible fungi as a substrate for the hydrolytic enzymes production using *Trichoderma* spp. found a xylanases production of 2.32 AU/mL and of cellulases of 0.76 AU/mL. In the present work, *T. harziamun* was able to grow on barley straw in the four treatments, it was observed that the highest cellulolytic and xilanolytic activities occurred in the treatment of unwashed barley straw supplemented with salts, it is very likely that *T. harzianum* is able to metabolize in the first instance, the carbohydrates, present in the substrate, that were not eliminated with the washing with water, and that once consumed, consume the hemicellulose and cellulose present in the barley straw. *T. harzianum* was able to grow on barley straw and in part, this behavior can be explained due to the presence of laccase enzyme, Chakroun et al. [34] described a laccase by *Trichoderma atroviride* produced in a liquid medium, Sadhasivam et al., [35] reported a laccase by *T. harzianum* also produced in a liquid medium. The laccases produced by *Trichoderma* have been applied to processes of biodegradation of dyes [36,37] and pulp bio-bleaching processes for paper [38] among others. It should be noted that the laccase enzyme has also been assigned an important role in defense processes against antagonistic organisms such as fungi and bacteria [39] and in sporulation processes [40].

As another part of the work, the addition and non-addition of salts, as well as the washing or not washing of barley straw on the conidiation process, were studied. According to these results, it is observed that washing or not washing the barley straw and adding and not adding salts have a significant effect on the conidiospores production. Washing with water applied to barley straw favors the extraction of soluble compounds such as starches, carbohydrates, tannins, gums, inorganic compounds, and coloring matter that could contain barley straw [18] therefore, *T. harzianum* probably have the enzymatic battery that allows it to survive on complex substrates and does not need soluble compounds (such as those extracted during the washing of the barley straw), it was also observed that the addition of salts favors the conidiospores production, it is important, therefore, the presence of some minerals such as K, Mg, Na, P, and S as well as N (present in the Pontecorvo salts) that together, allow an optimum development of the fungus and, therefore, a greater growth associated with greater sporulation. It has been described that environmental factors such as carbon source, nitrogen, C/N ratio, pH, calcium ion concentration as well as exposure to light can directly affect conidial processes [41], in the tests carried out, the pH remained around 5.9 despite the fact that it was not controlled. So this factor had no influence on the sporulation process. It has been described that alkaline pHs can have negative effects on sporulation of filamentous fungi such as *Trichoderma koningii* and *Penicillium chrysogenum* [42]. Jackson et al. [43] found that the absence of N, Mg, K, and P reduces the sporulation of *Trichodrema pseudokoningii* when it was grown on agar plates. It has been described that the nutritional requirements are essential for the growth and sporulation of filamentous fungi. In this way, Leite et al. [44] found that the nitrogen source is more important than the carbon source for the sporulation processes of filamentous fungi, on the other hand, Verma et al. [45] observed that the addition of glucose in the growth medium decreased the production of spores and the presence of a nitrogen source as meat peptone improved the production of spores. On the other hand, it has been described that complex carbon sources can also favor the sporulation of some filamentous fungi [46]. Another important factor that can favor the processes of sporulation is the C/N ratio, The analyzes performed on the initial substrate (barley straw) indicated a C/N ratio of 145:1, the C/N ratio has a strong impact on sporulation, has been described in *Trichoderma viride* that C/N ratios of 160:1 improve spore production, however, the effect of the C/N ratio in the sporulation is very varied since for example in *Metarhizium anisopliae* the greatest sporulation was observed in a C/N ratio of 20:1 [47].

Michel-Aceves et al. [9] described the massive production of *Trichoderma* spores using 15 organic substrates, they found the best results using corn cob with a production of 4.43×10^8 spores/mL and viability of 99 %. Harman et al. [48] tested indefinite culture media (means of potato dextrose, malt extract, and trypticasein soy) and defined (Czapek medium and minimum medium for *Neurospora*) obtaining 10^7-10^8 spores/g of matter. Deschamps et al. [27] used different proportions of wheat straw, bran, cassava, potato starch and sugar beet for the production of the spores, they found that the best medium was using the beet obtaining up to 7×10^9 spores/gdm attributing this yield, to the presence of available sugars. Verma et al. [45] using starch as a carbon source and under liquid growth conditions, obtained up to 4.2×10^7 CFU/mL of *T. viride* conidiospores.

The effect of the initial moisture of the substrate on sporulation was studied. The moisture of the substrate is very important for the growth of the fungus, appropriate moisture facilitates the development of mycelium and later, sporulation. Depending on the microorganism, the moisture requirements for an optimum development are different, for example, for the sporulation of *Beauveria bassiana*, the need for up to 90 % of the initial moisture of the substrate has been reported [49]. Flodman and Noureddini [50] cultivated *Trichoderma reesei* on maize grains coming from distilleries with an initial humidity of 50 %, they observed a conidiation of up to 7.5×10^8 spores/gdm after 136 h of cultivation in solid growth conditions with agitation mechanical, on the other hand, Rocky-Salimi and Hamidi-Esfahani [51] evaluated the growth of *T. reesei* on rice bran with an initial humidity of 70 % observing high hydrolytic activity.

One of the major limitations of the conservation processes of conidiospores is their viability during the storage period, depending on the conservation process will be the viability of the same. One of the most used conservation methods is dehydration because it provides greater stability and easier handling as well as storage capacity at room temperature [52] however, it has been described that the drying process reduces the survival of the conidiospores since the desiccation favors the oxidation of the lipids of the cell membrane affecting the survival of the spores [53]. The conidiospores viability by the drying method depends on the process, in this way it has been reported that the percentage of survival when *Metarhizium flavoviride* spores are dried only with dry air is 90 % once the process is finished [54], Pedreschi and Aguilera [52] reported 63 % survival when the spores are dried on silica gel at room temperature while Fernández-Sandoval et al. [53], report an 80 % survival rate with spray drying. The loss of viability reported is only during the conservation process, it should be taken into account the viability during storage, it is clear that drying at room temperature is the least aggressive. It is likely that conserving the conidiospores on the substrate increases the viability of the spores since it has been described that the presence of conidia improve the resistance of the spores to adverse environmental conditions [55], which is why it is recommended keep the spores adhered to the conidia on the substrate for its conservation and maintain its viability with respect to time.

5. Conclusions

The obtained results indicate that it is viable to use barley straw as a support and as a substrate for the production of conidiospores. It is important to pretreat the substrate because it may or may not favor the growth *of T. harzianum* due to the presence of soluble compounds that in an aqueous medium can be inhibitors of the growth of the fungus. The addition of salts increases the growth of *T. harzianum* as well as its sporulation, which is why it is necessary to supplement it with minerals that improve the development of the microorganism. The moisture is another important factor to take into account since adequate moisture throughout the growth of the fungus stimulates its growth and as a consequence, the increase in the number of conidiospores. Therefore, when taking care of these variables (washing, salts, and humidity), the barley straw becomes an excellent substrate for the production of *T. harzianum* conidiospores. Finally, maintaining the fungus on the substrate improvement the survival and viability of the conidiospores over time, so the system can be used simultaneously as a means of spore conservation.

Author statement

I send the corrections to the suggestions made by the reviewer 1 of the paper "Use of barley straw as a support for the production of conidiospores of *Trichoderma harzianum*". The corrections were made on the document which I send again.

CRediT authorship contribution statement

M.G. Serna-Díaz: Investigation, Methodology. **Y. Mercado-Flores:** Conceptualization, Validation. **A. Jiménez-González:** Conceptualization, Methodology. **M.A. Anducho-Reyes:** Methodology, Software. **J. Medina-Marín:** Validation, Formal analysis. **J.C. Seck Tuoh-Mora:** Validation, Formal analysis. **A. Téllez-Jurado:** Writing - original draft, Visualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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