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ECORFAN Journal Republic of Nicaragua

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Knowledge Area

The works must be unpublished and refer to topics of agriculture-forest, pathology-sustainable, forest, management, horticulture, engineering and integrated water useand other topics related to Biotechnology and Agricultural Sciences.

Presentation of Content

In the first article we present, *Reinvigoration of deteriorated corn seed with wetting and drying technique*, by ARELLANO-RODRÍGUEZ, Luis Javier, RODRÍGUEZ-GUZMÁN, Eduardo, CARRIZALES-MEJIA, Norberto and JIMÉNEZ-JIMÉNEZ, José Carlos, with ascription in the Universidad de Guadalajara, as the last article we present, *Biogas production from cattle manure and sugar cane tip (Saccharum officinarum)*, by GARCÍA-JONGUITUD, Karina, MOJICA-MESINAS, Cuitláhuac, VIDAL-BECERRA, Eleazar and ACOSTA-PINTOR, Dulce Carolina, with ascription in the Instituto Tecnológico de Ciudad Valles, as the last article we present, *Determination of protein of edible insects*, by GONZÁLEZ-AGUILAR, Delia, GALVÁN-LOZANO, Diana, PACHECO-GALLARDO, Carlos and CABRERA-DÍAZ, Elisa, with ascription in the Centro Universitario de Ciencias Biológicas y Agropecuarias, as the last article we present, *Influence of nitrogen and organic fertilization in the production of pungence in the crop of chile jalapeño (Capsicum annum)*, in greenhouse and open sky, by ARREGUIN-SOTO Javier, ZUÑIGA-MALDONADO, Walter Manuel and ORTEGA-GARCIA, Nicolas, with ascription in the Instituto Tecnológico Superior de Salvatierra, as the last article we present *Identify healthy and dead cells simultaneity of Bacillus subtilis MZ through to use of propidium iodide and acridine orange*, by HERRERA-REYES, Naieli, OLALDE-PORTUGAL, Víctor and SANCHEZ-SEGURA, Lino, with ascription in the Universidad Tecnológica del Norte de Aguascalientes.

Content

Article **ECORFAN Journal-Republic of Nicaragua**
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Reinvigoration of deteriorated corn seed with wetting and drying technique

Revigorización de semilla deteriorada de maíz con la técnica de humedecimiento y secado

ARELLANO-RODRÍGUEZ, Luis Javier†*, RODRÍGUEZ-GUZMÁN, Eduardo, CARRIZALES-MEJIA, Norberto and JIMÉNEZ-JIMÉNEZ, José Carlos

Universidad de Guadalajara. Departamento de Producción Agrícola. Centro Universitario de Ciencias Biológicas y Agropecuarias, Camino Ing. Ramón Padilla Sánchez 2100, Nextipac, 44600 Zapopan, Jalisco

ID 1st Autor: *Luis Javier, Arellano-Rodríguez* / **ORC ID**: 0000-0002-3188-0245, **arXiv Author ID**: LuisJavier#1, **CVU CONACYT ID**: 65995

ID 1st Coautor: *Eduardo, Rodríguez-Guzmán* / **ORC ID**: 0000-0002-4640-7610, **Researcher ID Thomson**: T-9496-2019, **CVU CONACYT ID**: 67847

ID 2nd Coautor: *Norberto, Carrizales-Mejia* / **ORC ID**: 0000-0003-4358-714X, **arXiv Author ID**: Norberto5506, **CVU CONACYT ID**: 255239

ID 3rd Coauthor: *José Carlos, Jiménez-Jiménez*

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Abstract

With the objective of reinvigorating damaged corn seed, the technique of wetting and drying the seed with five types of water and 10 imbibition times. Estimating the variables emergency percentage, emergency speed, seedling length and root length, and plant height 30 days emergency. For the variable speed of emergency for imbibition time the highest vigor corresponded to 18 and 12h, and with an emergency percentage of 62% higher in 10% of the control, it was 4, 8 and 18h. In water types for emergency percentage, the highest values were observed in artesian well and Key water. In seedling length with values of 25 to 24 cm were distilled water 30h, artesian well 8h y 18h; and the control was 22 cm. In radicle length the highest values corresponded to E. Pure® 24h and key water 14h. For plant height in 8h artesian well present a value of 175 cm, and the control with a value of 165 cm.

Reinvigoration, Corn, Wetting, Imbibition

Resumen

Con el objetivo de revigorizar semilla de maíz deteriorada, se utilizó la técnica de humedecimiento y secado de semilla con cinco tipos de agua y 10 tiempos de imbibición. Estimando las variables porcentaje de emergencia, velocidad de emergencia, longitud de plántula y longitud de raíz, y altura de planta a los 30 días de emergencia. Para la variable velocidad de emergencia en tiempo de imbibición el vigor más alto correspondió a 18 y 12h, y con un porcentaje de emergencia de 62% superior en 10% del testigo, fue de 4, 8 y 18h. En tipos de agua para porcentaje de emergencia los valores más altos se observaron en agua de la llave y agua de pozo. En longitud de plántula con valores de 25 a 24 cm se ubicaron el agua destilada 30h, agua de pozo 8h y 18h; y el testigo se ubicó con 22 cm. En longitud de radícula los valores más altos correspondieron al E. Pura® 24h y agua de la llave 14h. Para altura de planta en agua de pozo 8h presento un valor de 175 cm, y el testigo con un valor de 165 cm.

Revigorización, Maíz, Humedecimiento, Imbibición

Citation: ARELLANO-RODRÍGUEZ, Luis Javier, RODRÍGUEZ-GUZMÁN, Eduardo, CARRIZALES-MEJIA, Norberto and JIMÉNEZ-JIMÉNEZ, José Carlos. Reinvigoration of deteriorated corn seed with wetting and drying technique. ECORFAN Journal-Republic of Nicaragua. 2019. 5-9: 1-5

* Correspondence to Author (email: javier.arellano@academicos.udg.mx)
† Researcher contributing as first author.

Introduction

A quality seed is characterized by its high percentage of germination and vigor, which ensures the emergence in the field and the success in crop productivity; FAO recognizes that seeds occupy a central place in the agricultural development process: the improvement of seed quality is a fundamental factor in increasing the crop yield potential and is one of the most economical and efficient means to improve Agricultural production and productivity (FAO, 2006). The storage time of the seed and the high temperatures and high humidity prevailing during its conservation favor its deterioration. Studies carried out in soybean (Moreano et al., 2011) allow to maintain that the reduction in the physiological quality of the seed is a consequence of common problems such as aging and deterioration.

To counteract the negative effects of the seed deterioration process, various treatments have been used with partial success in various species, such as chemical treatment before sowing (pre-sowing) (Grzesik and Janas, 2014) and pre-hydration (pre-soaking) with water or with growth regulators this to improve germination capacity and vigor (Butola and Badola, 2004; Herrera et al., 2011). Seed germination can be defined as a series of metabolic and morphogenetic processes, which result in the transformation of the embryo into a seedling capable of becoming an adult plant. In the germination of the seed, sequential and synchronized processes occur and are recognized in such a way that anabolic and catabolic processes take place simultaneously. (Coll et al., 1995).

During the germination process the stage known as imbibition is presented. The imbibition is the process of water intake by the seed (Moreno et al., 2006). This occurs by immersing the seeds in osmotic solutions or in certain amounts of water for a certain period of time. The imbibition allows a greater number of seeds to quickly reach the same level of humidity and activate the metabolic apparatus related to the pre-germination process (Burgas and Powell, 1984). In this way, a physiological path known to improve the germinative behavior of many species of agricultural interest is the pregerminative treatments of wetting and drying of the seeds.

Which has proven to be efficient to reinvigorate aged seeds, accelerate and increase the germination and yields of plants, both under optimal and adverse ecological conditions (Bradford, 1986). Water is essential for the rehydration of the seeds. The amount of water absorbed by an imbibed seed depends on a number of factors, such as the size of the seed, permeability of the testa and the age of the seed. In absolute terms, water absorption is relatively small and can often not exceed 2 to 3 times the dry weight of the seed. For the establishment and subsequent development of the seedling, a greater and sustained water supply is required. Consider: 1) the water relations of the seed and 2) the relationship between the seed and its substrate (Bewley and Black 1994; Moreno et al., 2006). Thus, in the present study, there was a deteriorated corn seed, and to reinvigorate it, the following objective was raised.:

Objective

Reinvigorate damaged corn seed with the use of the seed wetting and drying technique using five types of water and 10 imbibition times.

Materials and methods

The experiment was carried out in experimental fields of the facilities of the University Center of Biological and Agricultural Sciences, in Las Agujas, Zapopan, Jalisco. A variety of yellow corn was used with an initial germination of 50%. And five types of water: 1) Ciel® Water (EC = 0.6 dS m-1 and pH = 5.8), 2) Epura® Water (EC = 0.4 dS m-1 and pH = 7.3), 3) Tap water (EC = 0.9 dS m-1 and pH = 7.6), 4) Distilled water (EC = 0.1 dS m-1 and pH = 8.5), and 5) Deep well water (EC = 3.0 dS m-1 and pH = 6.3) . The seed was imbibed in each type of water at times of 4, 8, 12, 18, 24, 30, 38, 44, 50 and 60 h respectively. After each imbibition period, the seed was dried for five days at room temperature and / in the shade.

The Imbibition Cup (IT) variable was created as a result of the difference between the initial weight and final weight (after each imbibition period) With the initial electrical conductivity (EC) and pH data and at each imbibition time the effects of these variables on the imbibition rate. The 51 treatments derived from water types (5) and times (10), plus the control (unimbibed seed) were sown under field conditions in three repetitions of 100 seeds each.

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Evaluating the emergency speed (VE) (Maguire, 1962). Data were taken daily of the number of seedlings emerged per plot / day and ended 15 days after planting. In this same test, the number of emerged seeds/plots was counted, resulting in the emergency percentage variable (% MS). On the other hand, the 51 treatments were sown at 25 seeds / plot every 10 cm and in three repetitions; evaluating the variables seedling length (LP), root length (LR), and plant height 30 days after the emergency (AP). The experiments were placed under a completely randomized block design, the results were processed with the SAS 2009 statistical package through analysis of variance, and Tukey test at 5% probability.

Results and Discussion

In the Imbibition Cup variable, no significant differences were obtained with the five types of water, but it did show significant differences ($\alpha \leq 0.05$) in imbibition times. In the variables electrical conductivity and pH, significant differences were found between types of water and imbibition times. In Table 1, it can be seen that at 60 h the highest IR and EC and the lowest pH were obtained.

Imbibition Time				Water Type		
IR		EC	pH		EC	pH
60 h	79 a	5.4a	3.7f	CIEL®	2.4b	4.9b
50 h	60 b	3.9b	3.9f	EPURA®	2.1b	4.7b
44 h	58 b	2.6c	4.4def	LLAVE	2.4b	4.5b
24 h	52 bc	2.6c	4.9cd	DEST	1.8b	5.5a
38 h	52 bc	2.5c	4.8cd	POZO	4.2a	4.8b
30 h	51 bc	2.5c	4.8cd			
18 h	43 cd	2.5c	4.4def			
12 h	40 de	2.3d	4.7cde			
8 h	32 ef	1.5cd	5.5bc			
4 h	24 f	1.4cd	5.8b			
In	0.0g	1.0d	7.1a			

Where: In = start, IR = Imbibition rate, EC = Electrical Conductivity. Means with the same letter in each column are statistically equal (Tukey $\alpha \leq 0.05$ <)

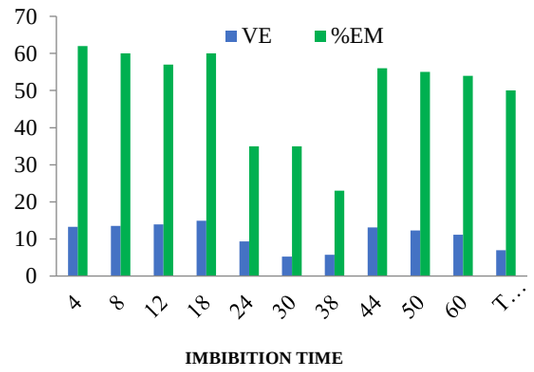
Table 1 Comparison of means for the variables of imbibition cup, electrical conductivity and pH, on imbibition time and types of water

Oguntunde and Adebawo (1989), determined the individual water absorption patterns of cv 'TZPB' corn seeds, local white corn, white and red sorghum types at 30, 35, 40 and 45 ° C soaked for 0 to 72 hours. The hydration curves obtained showed that the highest moisture absorption of the seeds of these materials occurred within the first 24 hours, while the maximum water absorption or moisture saturation content occurred after 36 hours, regardless of temperature in soaking.

For water type, the highest EC was presented in the deep well water, and the highest pH value (5.5) was obtained with distilled water (Table 1). The other treatments followed the same trend. For example, the distilled water at the beginning of the test had the lowest EC values (0.1 dS m-1) and the highest pH value (8.5), ending with values of 1.8 and 5.5 dSm-1 respectively at 60h of imbibition. The electrical conductivity test is based on the greater or lesser release of leachate by the seeds (ions, sugars, amino acids, among others) to the imbibition medium according to their physiological condition. The damaged seeds or with low physiological potential (less vigor) are those that release more leachate into the environment (greater conductivity), due to the loss of the integrity of cell membranes (Vieira et al., 2002).

In the variable emergency speed (VE); According to the analysis of variance, no significant differences were obtained for water types, but for imbibition times. While for% EM significant differences were found for times and types of water.

When performing the comparative test of means, the greatest vigor was obtained at 6 pm (14.9), followed by 12, 8, 4 and 44 hours respectively. And with lower values next to the control (seed without imbibir) 38 and 30 h (Graph 1). While the highest emergency percentage was obtained at 4, 8 and 18 h, surpassing the witness (50%) with 62% emergency (Graph 1).



Graphic 1 Behavior of the emergency speed (VE) and emergency percentage (% EM) variables based on 10 imbibition times

The treatment of 18 h of imbibition was observed as the one with the highest vigor and with a percentage that exceeded the control by 12%. Greatly favoring the recovery of the damaged seed.

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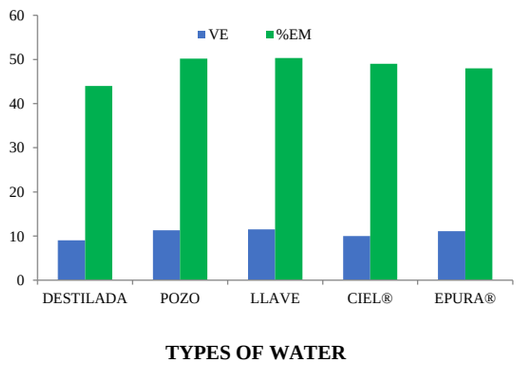
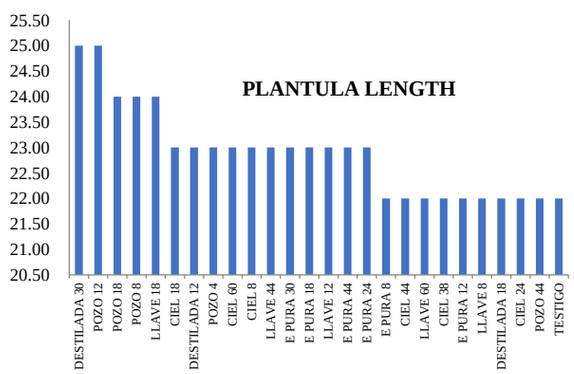
And it should be noted that the lowest electrical conductivity was observed in the times of 4, 8 and 18 h (Table 1). Times that favored the least amount of leachate in the water, and therefore the least deterioration. The electrical conductivity test (EC) and the pH test have been proposed to provide estimates of germination and / or vigor of seeds in 24 hours or less (Wilson, 1992). The range of electrical conductivity required for adequate crop growth is between 1.5 to 3.0 dS m-1, depending on the species and the EC of the water (Carrasco and Izquierdo, 1996).

In the comparative test of means, in types of water, it is observed in graph 2, that the lowest vigor was obtained in distilled water, While the highest percentage of emergency (% MS), was observed with the tap water and the deep well; followed by A. Ciel® and A. EPura ® (Graph 2).



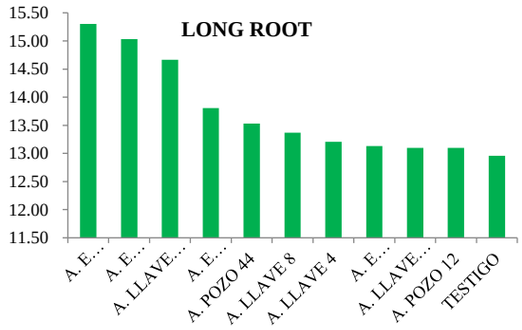
Graphic 2 Behavior of the emergency speed (VE) and emergency percentage (% EM) variables based on five types of water

For the seedling length variable, greater length was observed in the treatments of distilled water 30 h and well water of 12, 18 and 8 h respectively and the tap water at 18 h (Graph 3).



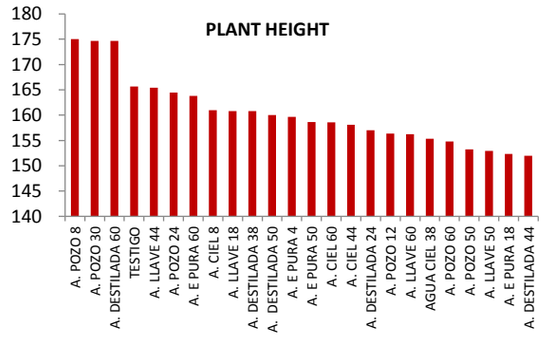
Graphic 3 Behavior of the seedling length variable (LP) in imbibition times and water types

In the variable root length (LR), the treatments that exceeded the behavior of the control (unimbibed seed), and were much higher than the rest: EPura® 24, 38, and 12 h respectively. As well as tap water with 24 h of imbibition (Graph 4).



Graphic 4 Behavior of the variable root length (LR) at imbibition times and water types

In the variable plant height, taken 30 days after the emergence of the seedling, only the deep well water treatments 8 and 30 h respectively and 60 h distilled water reached a height greater than 175 cm. In the control, a height of 165 cm higher than the rest of the treatments was observed (Graphic 5).



Graphic 5 Behavior of the variable plant height at 30 days after emergence in imbibition times and water types

Conclusions

In the five types of water the imbibition rate and electrical conductivity were increased as the imbibition periods increased. Otherwise the pH decreased. For the variable emergency speed at imbibition time, the highest vigor corresponded to 18 and 12 h, and with an emergency percentage greater than 60% it was 4, 8 and 18 h. Favoring these variables with the use of tap water and deep well water.

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25 treatments were superior in seedling length, and 10 treatments obtained the highest values of root length in relation to the control.

There was a significant effect on the plant height variable with the use of water types and imbibition times by showing increases and decreases in height in relation to the control.

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Biogas production from cattle manure and sugar cane tip *(Saccharum officinarum)*

Producción de biogás a partir de estiércol de ganado bovino y punta de caña de azúcar *(Saccharum officinarum)*

GARCÍA-JONGUITUD, Karina†, MOJICA-MESINAS, Cuitláhuac, VIDAL-BECERRA, Eleazar and ACOSTA-PINTOR, Dulce Carolina

Tecnológico Nacional de México. Instituto Tecnológico de Ciudad Valles

ID 1st Author: *Karina Lizeth, García-Jonguitud* / **ORC ID**: 0000-0001-8585-8249, **Researcher ID Thomson**: T-3267-2018, **arXiv Author ID**: GAJK, **CVU CONACYT ID**: 949371

ID 1st Coauthor: *Cuitláhuac, Mojica-Mesinas* / **ORC ID**: 0000-0001-8585-8249, **Researcher ID Thomson**: T-3267-2018, **arXiv Author ID**: MOMC640319JF8, **CVU CONACYT ID**: 744041

ID 2nd Coauthor: *Eleazar, Vidal-Becerra* / **ORC ID**: 0000-0003-3857-2103, **Researcher ID Thomson**: T-1547-2018, **arXiv Author ID**: elia.vidal, **CVU CONACYT ID**: 623037

ID 3rd Coauthor: *Dulce Carolina, Acosta-Pintor* / **ORC ID**: 0000-0003-0784-7039, **Researcher ID Thomson**: T-3349-2018, **arXiv Author ID**: DulceAcosta, **CVU CONACYT ID**: 626925

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Abstract

The purpose was to study the use of bovine manure mixed with sugarcane tip (*Saccharum officinarum*) in the production of biogas. A semicontinuous biodigester prototype of 1.7 m long and 6 in. diameter was designed and built with CPVC material. The waste was collected fresh for physical-chemical characterization, based on basic laboratory methods. Then the prototype was put into operation under controlled conditions. The biodigester was fed in two moments: the first, with 200 g of manure diluted in 750 ml of water and the second with a mixture of 200 g of manure and 50 g of cane tip, diluted in 750 ml of water. Finally, the volume of biogas produced and its quality were measured, with a water column pressure gauge and with a 540 multitec device, respectively. The results show that manure with the cane tip generates more biogas, this is attributed to the higher percentage of volatile solids in the mixture, whose organic components were converted to methane. The biogas production studied is a management option for waste from the agricultural sector and thus mitigate CO_2 emissions in the region.

Bovine manure, Sugar cane tip, Biogas

Resumen

El propósito fue estudiar el uso de estiércol bovino mezclado con punta de caña de azúcar (*Saccharum officinarum*) en la producción de biogás. Se diseñó y construyó un prototipo de biodigestor semicontinuo de 1.7 m. de largo y 6 pulg. de diámetro, con material CPVC. Se recolectaron en fresco los residuos para su caracterización físico-química, basada en métodos básicos de laboratorio. Después se puso en operación el prototipo bajo condiciones controladas. Se alimentó el biodigestor en dos momentos: el primero, con 200 g de estiércol diluido en 750 ml de agua y el segundo con una mezcla de 200 g de estiércol y 50 g de punta de caña, diluida en 750 ml de agua. Finalmente, se midieron el volumen producido de biogás y su calidad, con un manómetro de columna de agua y con un equipo multitec 540, respectivamente. Los resultados muestran que el estiércol con la punta de caña genera más biogás, esto se atribuye al mayor porcentaje de sólidos volátiles en la mezcla, cuyos componentes orgánicos se convirtieron en metano. La producción de biogás estudiada es una opción de manejo para los residuos del sector agropecuario y de esta manera mitigar las emisiones de CO_2 en la región.

Estiércol bovino, Punta de caña de azúcar, Biogás

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† Researcher contributing as first author.

Introducction

Currently the world is suffering from environmental deterioration due to climate change. This is because today man performs various activities that negatively impact the environment. Currently, according to data from the Mauna Loa observatory in Hawaii, the CO2 concentration recorded in the month of May this year, more than 415 ppm, much more than at any other time in human history (CO2.earth, 2019).

Some of the activities considered with the highest greenhouse gas emissions (methane and carbon dioxide) are those of the livestock sector, particularly the generation of manure that pollutes the soil, water and air, impacting the environment considerably (Varnero, 2011).

On the other hand, in agricultural production waste is also generated, as in the case of sugarcane with its high production of green and dry matter per unit area that translates into non-industrializable biomass (tips, leaves, sprouts, stems immature) that can be valued. Although these wastes are used for feeding cattle, their use is not total because they have low levels of protein, which do not cover the needs of ruminants, so they are used only to supplement diets (Galina, 2007).

The proper use and management of biomass is an important issue, as it is also considered susceptible to being transformed into alternative energy (García, 2016). It is necessary to study and know the useful resources that each region has in order to quantify the potential available for energy generation and contribute to national policies for the benefit of climate change and sustainable development of the planet (Acevedo and Rojas, 2017) .

According to Torres, 2016, sugar cane produces a large amount of biomass, composed in its state of maturity by 71.8% of stems, 12.6% of cane tips (buds), 8.7% of leaves and 6.9% of sprouts (Torres, 2006). In the State of San Luis Potosí, at the close of the 2016-2017 harvest, the production volume was 653 thousand tons of sugar, occupying the third national place. These data illustrate the large amount of cane tip that is available in the sugarcane region (Huasteca) as biomass for the production of biogas, considering its use in feeding cattle with the limitation that is not a total use.

In the country, goals for the mitigation of climate change have been established, there are also laws on energy matters that consider the incorporation of clean energy into the National Electricity Sector (SEN). While in the rural sector the use of biogas has generated interest in being an easily implemented technology (SENER, 2016). Biogas is mainly composed of a mixture of gases such as methane (CH4), carbon dioxide (CO2), hydrogen sulfide (H2S), among others. In addition, it is a gas that originates from the natural process of the decomposition of organic matter in the absence of air (FIRCO, 2010).

Therefore, the objective of this research was to study the use of manure from fresh cattle mixed with sugarcane tip (Saccharum officinarum) in the production of biogas. For this, a semi-continuous type CPVC biodigester was used, fed with the mentioned residues. The physical-chemical characterization was carried out and the quantity and quality of the biogas generated from them was evaluated.

Methodology

This research was carried out in the Chemistry Laboratory of the Technological Institute of Ciudad Valles, located in Ciudad Valles, S.L.P, Mexico. The Municipality of Ciudad Valles, is located to the East of the state of San Luis Potosí, in the Huasteca region. It is at an average height of 200 meters above sea level. Its climate is hot humid; in spring and summer times, maximum temperatures of up to 50 ° C are presented and in winter there are minimum temperatures of 6 to 8 ° C with a humid cold.

According to the objectives set forth in this paper, an investigation was carried out that conforms to the experimental and explanatory type. It is considered experimental because the organic waste (manure and cane tip) was subjected to the biodigestion process in order to study the production of biogas from them; and it is considered explanatory because of the need to look for cause and effect relationships to explain why the results. A work strategy was designed, which consisted of four phases:

Sample collection

Fresh samples of cattle manure and cane tip were collected for the study, in a cattle ranch and cane of Ciudad Valles.

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The samples were collected every week, the material was kept refrigerated, and from there the waste was prepared to feed the biodigester daily.

Physical-chemical analysis of manure and manure with sugarcane tip

The samples were tested to identify the conformation of the residues, as illustrated in Figure 1. These tests included basic laboratory analysis methods based on the procedures of the respective Official Mexican Standards. The tests performed were moisture, ash, and total nitrogen. Likewise, the percentages of total solids, volatile solids, organic carbon and the C / N ratio were calculated. A sample with three repetitions of 5 g was taken from each residue mixture, to perform the corresponding analyzes, which are described below:

1) Humidity: As established by NMX-F-083-1986, samples were introduced in a drying oven at 105 ° C, until constant weight was obtained between two consecutive weighings. To calculate the moisture percentage, equation 1 was applied:

$$\% \text{ moisture} = \frac{(B-A)-(C-A)}{B-A} \text{x}100 \quad (1)$$

Where:
A = Weight of the bottle at constant weight (g)
B = Bottle weight at constant weight with wet sample (g)
C = Weight of the bottle with dry sample (g)

Figure 1 Moisture determination of organic waste
Own Source

2) Total solids (St): They were obtained by difference, with respect to the percentage of humidity. The content of ashes and volatile solids was determined.

Ashes: This test was carried out using NMX-F-066-S-1978, where 2 g of the wastes were taken from which the humidity was determined and the ashes percentages were calculated. This percentage was obtained by difference in weights, using equation 2:

$$\% \text{ of ashes} = \frac{(Crucible\ weight + ash) - (crucible\ weight)}{Sample\ weight} \text{x}100 \quad (2)$$

Volatile solids (Sv): Once the percentage of ashes was determined, the percentage of volatile solids was calculated by difference.

Total nitrogen: It was determined by the Kjeldahl method, based on the destruction of organic matter with concentrated sulfuric acid. Due to chemical reactions, ammonia is released, which is recovered by distillation and receiving it in sulfuric acid. Upon reacting, ammonium sulfate is formed, the excess acid is titrated (titrated), with sodium hydroxide using as a methyl red indicator. (NOM-F-68-S-1980).

C / N Ratio: The percentage of Organic Carbon was calculated from the percentages of organic matter (volatile solids). C / N ratios were calculated for each of the samples, using equations 3 and 4:

$$\% \text{ organic coal} = \frac{\% \, organic\ matter}{1.724} \quad (3)$$

Where:
1.724= Conversion factor

$$\text{C/N} = \frac{\% \, Organic\ Carbon}{\% \, of\ total\ nitrogen} \quad (4)$$

Where C / N = Carbon Nitrogen Ratio

Biodigester Operation

Because there is only one biodigester prototype, the study was done in two moments and under laboratory conditions with a controlled temperature of 26 ° C. In the first moment the biodigester was fed for 50 days with fresh manure, diluted in water in a 1: 3 ratio. In a second moment, the biodigester was fed for 50 days with manure and sugarcane tip, diluted in water in a 1: 4: 15 ratio, as shown in Table 1.

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Organic matter used	Water	Manure	Sugarcane Tip
Manure	750 ml.	250 g.	0 g.
Manure with sugarcane tip	750 ml.	200 g.	50 g.

Table 1 Quantities of organic matter introduced into the biodigester
Own Source

Once the samples were taken, they were homogenized from the amounts expressed in Table 1, and for this purpose a blender was used that allowed the materials to be fully incorporated. The mixtures were poured into the digester daily and in both cases (manure alone and manure mixed with sugarcane tip) the feeding time was 50 days. See figure 2.

Figure 2 Feed to the digester with the mixture of organic waste
Own Source

Study of biogas production

During the operation of the biodigester were measured: days of operation, days of feeding, days without biogas production, days with biogas production, days of biogas production without food and the volume of biogas produced. The latter was estimated daily with the support of a water column manometer that uses the principle of water displacement under the ideal gas law. Finally, in both cases the quality of biogas produced with the multitec 540 equipment (methane concentration, CO2 and H2S) was measured, as shown in Figure 3 and the results are shown in Table 4.

Figure 3 Measurement of biogas quality
Own Source

After the measurements, the generated methane gas was burned, using a Bunsen burner.

Results

Physicochemical characteristics of organic waste

In the present study the physicochemical characteristics of organic waste were determined, the results are shown in Table 2.

Physical-chemical test	Fresh manure		Manure with sugarcane tip	
	Obtained	Theoretical (Varnero, 2011)	Obtained	Theoretical (Varnero, 2011)
% Humidity	89.15	85-51	87.55	ND
% Sól. T	10.85	13.4-56.2	12.45	ND
% Ash	12.8	23.67	8.12	ND
% Sól. V	87.2	76.33	91.88	ND
% Carbon	22	25	22.88	ND
% N	1	1.5	.915	ND
C/N	22:1	25:1	25:1	ND

Table 2 Results of the physical-chemical tests performed on organic matter
Own Source

In the chemical analysis it was obtained that of the percentage of total solids shown in Table 2, the amount of volatile solids in the two samples (fresh bovine manure and cane-tipped manure) was above the theoretical reference amount that was 76.33% and thus, below the percentage of ashes. In this case, 87.2% volatile solids were obtained in the sample of bovine manure and 91.88% was obtained in the sample of mixed manure with cane tip.

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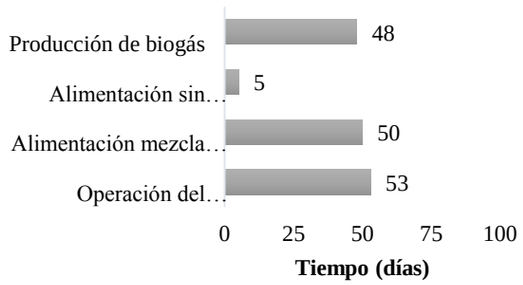
This indicates that when manure is mixed with the tip of sugarcane there may be a greater amount of methane because the percentage of volatile solids is higher, the organic compounds they contain are converted to methane.

Additionally, in determining the Carbon / Nitrogen ratio of the residues studied, Varnero (2011), reports that the optimal ratio for the methanogenic process to be carried out is 25/1. In the present study, the C / N ratio determined in fresh bovine manure was 22: 1 and in sugarcane-dung manure it was 25: 1. These results show that the mixture of manure with the tip of sugarcane has an optimal C / N ratio for the decomposition of organic matter, being present in appropriate proportions for a good feed of methanogenic bacteria.

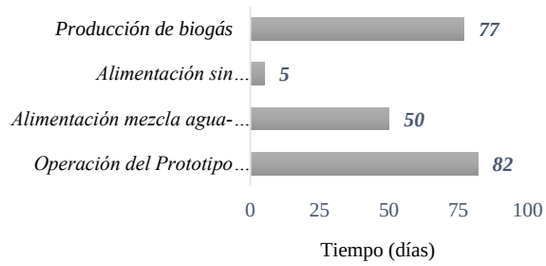
Study of biogas production

In this study, the times and volumes of biogas production were measured both in manure and in manure mixed with sugar cane tip. The measurements of the various activities during the operation of the digester are shown in Graphs 1 and 2.



**Laboratory controlled temperature of 26 ° C*

Graphic 1 Operating times of the laboratory biodigester prototype (water-bovine manure)
Own Source



**Laboratory controlled temperature of 26 ° C*

Graphic 2 Operating times of the biodigester prototype in the laboratory (water-bovine manure-cane tip)
Own Source

Thus, the production of biogas expressed in Table 3 illustrates that there was a greater volume of biogas during the time of operation of the digester, when the manure was mixed with the tip of sugarcane, obtaining 203% more than when only manure was used. This is explained by the greater amount of volatile solids that were obtained in the mixture of manure with the cane tip (91.88%) and the good C / N ratio, which indicates a greater presence of organic components than during the process of biodigestion with the work of methanogenic bacteria became methane.

Fresh manure		Manure with sugarcane tip	
Average biogas production per day (l)	Volume of biogas produced (l)	Average biogas production per day (l)	Volume of biogas produced (l)
3.82	183.23	4.85	373.61

Table 3 Biogas production
Own source

Likewise, with respect to the quality of biogas, this was better when the manure was mixed with the cane tip, since here a higher concentration of methane was obtained and consequently less CO_2 and H_2S, as can be seen in Table 4 The above is attributed to the best conditions for the biodigestion process present in the manure mixture with cane tip, derived from the C / N ratio.

	Biogas quality		
Residue Organic	Methane (CH_4)	Carbon dioxide (CO_2)	Hydrogen Sulfide (H_2S)
Fresh cattle manure	61.48%	37.40%	1.125%
Manure with sugarcane tip	66.1%	32.9%	1.0%

Table 4 Biogas quality obtained
Own Source

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Conclusions

The amount and composition of the biogas obtained through the biodigestion process depended largely on the digested material. In the present investigation a greater amount of biogas (373.61 l.) Was obtained when the manure was mixed with sugarcane tip, compared to when only manure was used (183.23 l.).

These results are explained by the higher percentage of volatile solids obtained when the cane tip was added, whose organic components were converted to methane. In addition, a better C / N ratio of 25: 1 was obtained, being an optimal ratio for the decomposition of organic matter, being present in appropriate proportions for a good feed of methanogenic bacteria that favored methane quality.

Due to the important sugarcane and livestock activity in Huasteca Potosina, it is recommended from this study, to quantify the energy potential of agricultural waste that allows us to project the capacity of usable energy in the region through biomass technologies. The above, in order to promote the stability of production systems based on the sustainable use of natural resources, so that the needs of future generations are not compromised.

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Determination of protein of edible insects

Determinación de proteína de insectos comestibles

GONZÁLEZ-AGUILAR, Delia†*, GALVÁN-LOZANO, Diana, PACHECO-GALLARDO, Carlos and CABRERA-DÍAZ, Elisa

Centro Universitario de Ciencias Biológicas y Agropecuarias. Camino Ing. Ramón Padilla Sánchez No. 2100, La Venta del Astillero, Zapopan, Jalisco. C.P. 45510

ID 1st Author: *Delia, González-Aguilar* / **ORC ID**: 0000-0003-1413-2330

ID 1st Coauthor: *Diana, Galván-Lozano* / **ORC ID**: 0000-0002-7720-5819

ID 2nd Coauthor: *Carlos, Pacheco-Gallardo* / **ORC ID**: 0000-0002-5851-4616

ID 3rd Coauthor: *Elisa, Cabrera-Díaz* / **ORC ID**: 0000-0002-1970-2104

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Abstract

Mexico is a country with a wide variety of edible insects. Because of its high protein intake, insect consumption is proposed as a solution to hunger. However, studies on nutritional value are necessary.The objective of this work was the determination of chapulin protein. In this study, 14 chapulin (*Sphenarium purpuarascens*) samples collected in Guadalajara from Oaxaca and Morelos were analyzed and processed according to the Weende proximate analysis methodology that includes the Kjeldahl procedure for protein determination. The results on fresh weight basis were: water 22.68%, dry matter 77.32%, ashes 12.8%, fat 6.78%, fiber 3.38%, protein 33.15% and nitrogen free extract 21.21%. Protein digestibility was 91.21%. Because of the protein content, chapulin flour can be used as a protein ingredient in other food products.

Insects, Nutritional value, Protein

Resumen

México es un país que cuenta con una gran variedad de insectos comestibles. Debido a su alto aporte proteico, el consumo de insectos se propone como solución del hambre. El objetivo de este trabajo fue la determinación de proteína del insectos, chapulín (*Sphenarium purpuarascens*). Se analizaron 14 muestras de chapulín recolectados en Guadalajara, Jal. Procedentes de Oaxaca y Morelos, y se procesaron de acuerdo a la metodología de los análisis proximales Weende que incluye el procedimiento de Kjeldahl para determinación de proteína. Los resultados en base húmeda (BH) obtenidos fueron: Humedad 22.68%, Materia seca 77.32%, cenizas 12.8%, grasa cruda 6.78%, fibra cruda 3.38%, proteína cruda 33.15% y extracto libre de nitrógeno (ELN) 21.21%. La digestibilidad proteica fue de 91.21 %. Por el contenido de proteína, la harina de chapulín, se podría utilizar como ingrediente proteico en productos alimenticios.

Insectos, Valor nutricional, Proteína

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† Researcher contributing as first author

Introduction

The supply of animal protein to a constantly increasing population represents an important challenge worldwide. Therefore, different solutions have been proposed, including encouraging the consumption of insects that are high in protein. Mexico is a country that has a wide variety of edible insects. Due to its high protein intake, insect consumption is proposed as a solution to hunger. However, studies on nutritional value are necessary. Currently, the consumption of insects, known as entomophagy, is practiced by more than 2,000 million people mainly in the regions of Asia, Africa and Latin America.

In the world there are more than 1,900 species of insects that are consumed by humans, among which are crickets, grasshoppers, escamoles, beetles, chicatanas, etc. (García V., 2018; Van Huis et al., 2013). In Mexico approximately 200 species are consumed, such as chapulines or crickets, maguey worms, escamoles (known as Mexican caviar and precious since pre-Hispanic times), the acociles and jumiles (Badui, 2015).

This has environmental, health and social benefits, such as high efficiency of food conversion, emit less greenhouse gases and require less land and water for their upbringing (Van Huis et al., 2013). Entomophageal practice is considered a viable food option by 2050, in which it is estimated that there will be 9,000 million inhabitants (Badui, 2015). However, in some countries such as the United States and Canada they present a rejection towards their consumption (García V., 2018).

Currently in Mexico there are several communities that have in their diet the consumption of insects, Oaxaca being the largest consumer, followed by states such as Guerrero, Morelos, Hidalgo, Chiapas, Veracruz and the State of Mexico, and with less in Campeche, Tabasco , Puebla, Querétaro, Guanajuato, Jalisco and Michoacán. Edible insects are a good source of protein, fat, unsaturated fatty acids, minerals of great importance such as iron and zinc, and vitamins such as thiamine and riboflavin (DeFoliart, 1997). To know the nutritional quality of insects it is necessary to determine the amount of protein and true moisture, fiber and raw fat, ashes, nitrogen-free extract, carbohydrates.

On the other hand, it is also necessary to know the amount of minerals such as magnesium, potassium, sodium, iron and zinc. The amount of protein in insects varies depending on the species and whether it is terrestrial or aquatic, among other factors. Most species have a proportion ranging from 55% to 70%. They are also rich in essential amino acids (Ramos E. 2007).

In Mexico, among the most consumed insects are the chapulines. They contain between 70 and 77% of protein, more than 50% of beef (Viesca G., 2009) and 62% of total protein and of this 89% is digestible (Ramos, E., 1989, Ramos, E. and Pino M. 1981). Since the consumption of chapulin is common in Mexico, studies on its quality and safety are necessary. The objective of the present investigation was to determine the protein value of Chapulin (Sphenarium purpurascencs).

Methodology

In the present study, 14 chapulin samples (Sphenarium purpurascencs) collected in Guadalajara, Jal., From the states of Oaxaca and Morelos were analyzed. Insects were obtained from suppliers with fixed establishments. They were transported in plastic bags labeled to the Laboratory of Food Physicochemistry of the Department of Public Health of the University Center of Biological and Agricultural Sciences of the University of Guadalajara where they were processed according to the methodology of Weende proximal analyzes that includes the Kjeldahl procedure for protein determination. The analyzes were performed in duplicate and the average values were reported. The determinations that were made were: moisture and dry matter, ashes, ethereal extract, fiber and crude protein, nitrogen-free extract (ELN) and protein digestibility. (Helrich, 1990).

Determination of moisture and dry matter

The moisture determination is based on the evaporation of water. In aluminum trays the chapulín samples were placed and left in a bacteriological oven at a temperature of 100 ° C for 18 hours, then the whole sample was pulverized in a mill. 5 g of each sample was taken and passed to the drying bell for 20 minutes and the rest was reserved for the following determinations.

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By weight difference, initial and final, the amount of moisture in the sample was calculated according to the following formula:

% dry matter = (residue weight) / (sample weight) x 100

% humidity = 100 -% dry matter

Ash Determination

Ashes is the inorganic portion that is obtained by calcining the food at temperatures between 550-600 ° C. In a crucible, 2 g of sample were weighed, the muffle was calcined at a temperature between 550-600 ° C for 3 hours (Fig. 1 and 2). At the end of this time, the temperature was expected to drop to 100 ° C to pass the crucible to the drying bell for 20 minutes and then the residue was weighed. To calculate the amount of ashes the following formula was used:

% ash = (residue weight) / (sample weight) x 100

Figure 1 Determination of ashes
Source: Self Made

Crude protein determination

The basis of the method for determining crude protein was to quantify the total nitrogen and multiply it by a certain factor, the factor suggested for edible insects was used by Janssen, et al. (2017). The samples were processed according to the method of the Kjeldahl method which consisted of quantifying the total nitrogen and multiplying it by a certain factor, the factor suggested by Janssen, et al. (2017). Thus, the percentage of Crude Protein (total) of an organic sample can be obtained (Fig. 2).

The percentage of nitrogen was calculated as follows:

% nitrogen = ml x normal HCL (0.1) x Meq. Nitrogen (0.014) / sample weight x 100

% crude protein =% nitrogen x 5.6

Figure 2 Determination of crude protein by the Kjedahl method
Source: Self Made

Crude Fat Determination

The Soxhlet method for determining crude fat (Fig. 3), quantifies the extractable substances in petroleum ether. 2 g of sample were weighed on filter paper, passed into a filter paper thimble and a glass for determination of crude fat. In the Soxhlet apparatus, extraction was carried out for 3 hours at a condensation rate of 5 to 6 drops per second. The ether was recovered and the residue was dried in a bacteriological oven at 100 ° C for 30 minutes. The samples were passed to the desiccator 20 minutes and the residue was weighed. The formula for calculating the percentage of raw fat is as follows:

% ethereal extract (G. C.) = (weight of the residue) / (weight of the sample) x 100

Figure 3 Soxhlet apparatus for determination of raw fat
Source: Self Mad

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Crude Fiber Determination

The method to determine the raw fiber was to calculate the loss by calcination of the residue of the acid and alkaline digestions of the sample (Fig. 4). For acid digestion, 0.5 g of each chapulin sample were weighed in previously degreased bags, transferred to a 600 ml Berzelius glass, 200 ml of 1.25% sulfuric acid was added, the vessel was placed in the digester and boiled for 55 minutes The same procedure is repeated for alkaline digestion, but with 200 ml of 1.25% sodium hydroxide. The procedure was continued according to the method specification. The crude fiber was calculated with the following formula. % crude fiber = (crucible weight + dry fiber) - (crucible weight + residue) - (bag weight * correction factor [0.992]) / sample weight x 100

Figure 4 Determination of crude fiber
Source: Self Made

Determination of Nitrogen Free Extract

To determine the Nitrogen Free Extract, carbohydrates, sugars and starches were calculated by subtracting the difference from the other determinations made, according to the following formula:

% Nitrogen-free extract = 100 - (% humidity +% ash +% crude fat +% crude fiber +% crude protein).

In vitro protein digestibility determination

3 g of sample were weighed and fat extraction was carried out, placed in a bacteriological oven at 100 ° C for 1.5 hours.

Two samples of 0.9859 g and 1.0777 g were weighed, each placed in a different Erlenmeyer flask labeled as sample 1 and sample 2, a solution of 3 ml HCL, 1 g pepsin, 50 ml of double distilled water was added to each flask and a magnet They were placed on a hot plate with stirring at a temperature of 39 ° C for 18 hours, filtered with a medium filtration filter paper in a vacuum pump. For each sample, the crude protein determination is performed and digestibility is calculated as follows:

Average digestibility =% crude protein sample 1 +% crude protein sample 2/2.

% digestibility =% crude protein -% average digestibility / average crude protein x 100

Results

The results of the proximal analysis in wet base (BH), which represents the value as it is in the chapulin samples can be observed in table No.1 and in table No. 2 the results in dry base (BS), that is, in samples without water.

Analysis	Value %
Dry material	84.37
Humidity	15.63
Crude protein	35.80
Ethereal extract	7.46
Ashes	14.47
Raw fiber	3.84
Nitrogen free extract	22.81

Table 1 Proximal chemical analysis Wet Base (B.H.) of Chapulín Sphenarium purpurascencs (grs / 100 g sample)
Source: Own Elaboration

Analysis	Value %
Dry material	100
Humidity	0
Crude protein	42.88
Ethereal extract	8.36
Ashes	17.96
Raw fiber	4.61
Nitrogen free extract	26.30

Table 2 Proximal chemical analysis Base Seca (B. S.) of Chapulín Sphenarium purpurascencs (grs / 100 g sample)
Source: Own Elaboration

On a wet basis, the protein digestibility of the samples was 91.21%.

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This data is similar to 89.63% reported by Ramos, E. and Pino M. (1981) and published by Aragón, G. (2018), whose values varied between 80.22 and 85.48%.

In this study, the amount of crude protein on a dry basis was 42.8% which was lower than the values between 52.13% and 75.3% published by Ramos, E. (1987).

Acknowledgments

We thank the staff of the Food Physicochemical Laboratory of the Department of Public Health of the University Center of Biological and Agricultural Sciences of the University of Guadalajara for the facilities granted to carry out the determinations of moisture and dry matter, ashes, ethereal extract, fiber and crude protein, nitrogen free extract (ELN) and protein digestibility.

Conclusions

Chapulín because of its high protein content can be an economic source and an important part in the diet of the human population.

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Article **ECORFAN Journal-Republic of Nicaragua**
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Influence of nitrogen and organic fertilization in the production of pungence in the crop of chile jalapeño (*Capsicum annum*), in greenhouse and open sky

Influencia de la fertilización nitrogenada y orgánica en la producción de pungencia en el cultivo de chile jalapeño (*Capsicum annum*), en invernadero y en cielo abierto

ARREGUIN-SOTO Javier†*, ZUÑIGA-MALDONADO, Walter Manuel and ORTEGA-GARCIA, Nicolas

Innovación Agrícola Sustentable, Instituto Tecnológico Superior de Salvatierra, Manuel Gómez Morín 300, col. Janicho, 38900, Salvatierra, Guanajuato, México, Departamento de Posgrado e Investigación

ID 1st Author: *Javier, Arreguin-Soto* / **ORC ID**: 0000-0003-2204-0209, **Researcher ID Thomson**: X-3004-2018, **CVU CONACYT ID**: 143848

ID 1st Coauthor: *Walter Manuel, Zuñiga-Maldonado*

ID 2nd Coauthor: *Nicolas, Ortega-Garcia*

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Abstract

Capsaicin and dihydrocapsaicin are responsible for 90% of the pungency of the fruit; the rest of the capsaicinoids participate to a lesser extent in the itching, but contribute strongly to the diversity of spicy flavors in the different species of Capsicum. In the present project the application of 7 treatment T1 = Chemical, T2 = Chemical + Bioferment, T3 = Chemical + Leached, T4 = Bioferment, T5 = Leached, T6 = Bioferment + Leached, T7 = Chemical + Leached + Bioferment, in greenhouse was evaluated and in open sky, a randomized block design with three repetitions was established. The variables evaluated were plant height, temperature, relative humidity, production weight, fresh weight, root depth, dry weight and pH. The data generated in this research indicate that the treatment that generated the most amount of capsaicinoids was the chemical treatment in the greenhouse with 37.7 ml of capsaicinoids and in the open sky with 36.96 ml.

Organic Fertilization, Chemical Fertilization, Pungence

Resumen

La capsaicina y la dihidrocapsaicina son responsables de 90% de la pungencia del fruto; el resto de los capsaicinoides participan en menor medida en el picor, pero contribuyen fuertemente a la diversidad de sabores picantes en las diferentes especies de Capsicum. En el presente proyecto se evaluó la aplicación de 7 tratamiento T1= Químico, T2=Químico+ Biofermento, T3= Químico + Lixiviado, T4= Biofermento, T5= Lixiviado, T6= Biofermento + Lixiviado, T7= Químico + Lixiviado + Biofermento, en invernadero y en cielo abierto, se estableció un diseño de bloques al azar con tres repeticiones. Las variables evaluadas fueron altura de planta, temperatura, humedad relativa, peso de producción, peso fresco, profundidad de raíz, peso seco y pH. Los datos generados en esta investigación, indican que el tratamiento que genero mayor cantidad de capsaicinoides fue el tratamiento químico en invernadero con 37.7 ml de capsaicinoides y en cielo abierto con 36.96 ml.

Organic fertilization, Chemical Fertilization, Pungence

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† Researcher contributing as first author

Introduction

In Mexico there are more than 100 varieties of chili, the most common of which are green chili, habanero, bell pepper, jalapeño and poblano chili. It should be noted that the production of chili in the country is considered one of the most important primary economic activities, since each year its production generates more than 22 billion pesos, which in addition to benefiting the economy of more than 12 thousand producers in Mexico (Muñoz. 2017).

The consumption of chili is mainly due to its spicy taste, caused by the presence of capsaicinoids, a group of acid amides derived from vanillylamine, which are synthesized and accumulated in the placental tissue. Different capsicum species may vary in degree of itching, which is related to their ability to accumulate capsaicinoids. The habanero chile (C. chinense) is considered the hottest; However, some varieties of C. annuum can reach similar levels, depending on the conditions in which they are grown (Vázquez. 2007).

Fertilization should be done precisely, planned and above all avoiding the use of compounds and / or products other than those indicated for the type of crop, soil, climate, etc. To cover effectiveness, organic and chemical fertilization should be taken into account as an economically and ecologically feasible alternative for production, in which there is a better use of nutrients and that they are available efficiently and quickly for plants (Barrales. 2010).

Problem Statement

Pungency is the spicy taste of the fruits of the Capsicum genus, and this is due to the presence of compounds called capsaicinoids that are found mainly in the placental tissue, however the presence of these compounds is affected by various factors such as environment and fertilization. The pungency in chili is due to the organic compound called capsaicin having great importance not only because it is the substance that causes the itching (pungency) in the chili, but also of vital importance since it is used for food preservation, it has anti -Microbial and anti-fungal, inhibiting the development of certain pathogens.

This is why the alternative of open sky and greenhouse fertilization is sought to identify if they influence the production of pungency in greater quantity, in order to provide sufficient quantity to the use in the capsaicin industry.

Objectives

General

Evaluate the influence of organic and nitrogen fertilization on the pungency of jalapeño pepper in the open sky and greenhouse

Specific

– Determine the pungency content of jalapeno pepper treated with 7 treatments
– Evaluate the effect that organic and chemical fertilization has on the pungency of open-pit jalapeño pepper and greenhouse

Methodology

The experiment was established under an experimental design by random blocks, where seven treatments with three repetitions will be evaluated.

Where the treatments are:

T1 = Chemical
T2 = Chemical + Bioferment
T3 = Chemical + Leaching
T4 = Bioferment
T5 = Leached
T6 = Bioferment + Leaching
T7 = Chemical + Leaching + Bioferment

T7, R1	T5,R2	T2, R1
T3, R2	T7, R3	T5, R1
T1, R1	T1, R3	T2, R3
T6, R1	T4, R3	T5, R3
T2, R2	T6, R2	T3, R3
T1, R2	T3, R1	T7, R2
T4, R2	T6, R3	T4, R1

Table 1 Experimental design for open sky and greenhouse

Contribution

Organic fertilization in a profitable alternative if the producer wants to obtain greater production, and for greater pungency the application of chemical fertilizer is recommended

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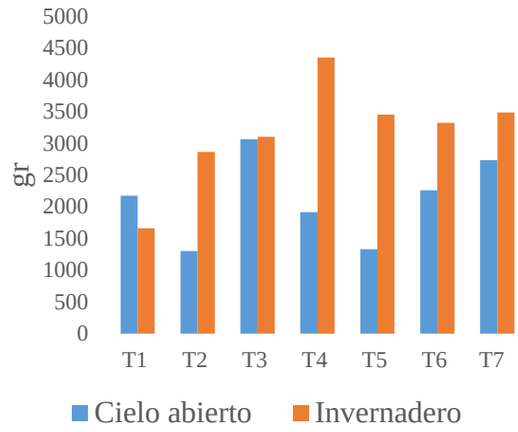
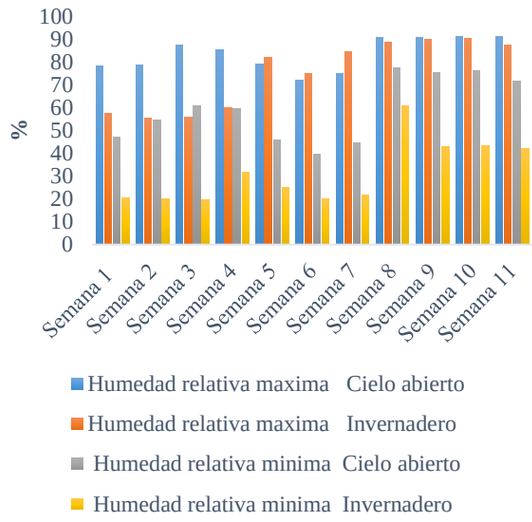
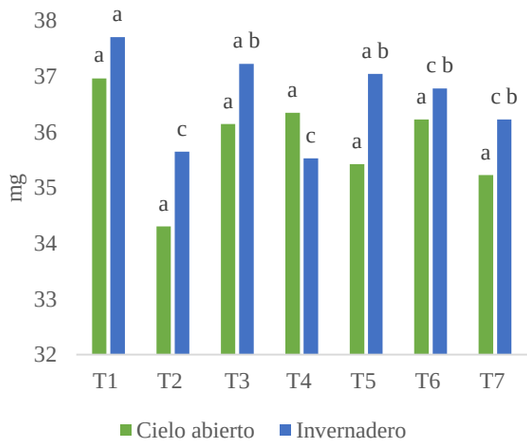


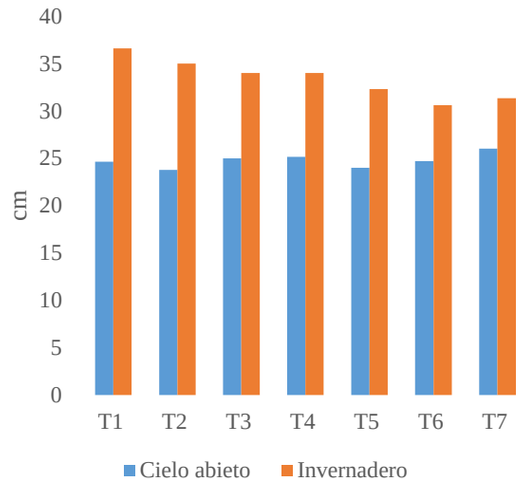
Graphic 1 Weight of production in open sky and greenhouse



Graphic 2 Record of temperature and humidity in open sky and greenhouse



Graphic 3 Capsaicinoids



Graphic 4 Plant height

Conclusions

For the height variable, the treatments established in the greenhouse had a different behavior having a greater height than the treatments established in the open sky.

According to the root depth variable, treatments T4 (B), T2 (Q + B) and T6 (B + L) showed a greater difference, being those established in the greenhouse those that presented larger roots, while treatments T3 (Q + L), T4 (B), T5 (L) and T7 (Q + L + B) showed no difference because they behaved similarly both in the greenhouse and in the open sky having shorter roots.

According to the pungency the treatments established in the open sky had no significant difference according to the Tukey test (P> 0.05). On the other hand, in greenhouse the treatments T1, T2 and T4 had a difference, with T1 being the one with the highest amount of capsaicinoids with 37.7 and the one with the lowest capsaicinoids was T4 with a total of 35.52.

With the data obtained in this investigation, it is concluded that the treatment that gave the best result in both greenhouse and open sky was the chemical treatment since it gave greater pungency, this means that the bioferment and leaching do not influence the production of capsaicin are however the temperature and relative humidity if since in greenhouse conditions the levels of pungency were greater than the levels of pungency in the open sky.

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Recommendations

Organic fertilization in a profitable alternative if the producer wants to obtain greater production, and for greater pungency the application of chemical fertilizer is recommended

The optimal conditions for the production of capsaicin are in the greenhouse since there are adequate temperatures and humidity for its production and with the proper fertilization better results are obtained.

It is recommended to repeat the experiment and thus corroborate the results obtained and improve the information on the production of capsaicin since it is an alternative of great potential for the farmer.

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Identify healthy and dead cells simultaneity of *Bacillus subtilis* MZ through to use of propidium iodide and acridine orange

Identificación de células vivas y células muertas simultáneamente de *Bacillus subtilis* MZ mediante el empleo de ioduro de propidio y naranja de acridina

HERRERA-REYES, Naieli†*, OLALDE-PORTUGAL, Víctor and SANCHEZ-SEGURA, Lino

Universidad Tecnológica del Norte de Aguascalientes, Departamento de Ingenierías, Aguascalientes, México

ID 1st Author: *Naieli, Herrera-Reyes* / **ORC ID**: 0000-0002-7654-1167, **CVU CONACYT ID**: 564450

ID 1st Coauthor: *Víctor, Olalde-Portugal* / **ORC ID**: 0000-0002-0795-6035, **CVU CONACYT ID**: 5556

ID 2nd Coauthor: *Lino, Sanchez-Segura* / **ORC ID**: 0000-0001-6179-3233, **CVU CONACYT ID**: 205788

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Abstract

The use of microorganism in a lot of process brings the development of new technologies. For guarantee the effect and the impact of the microorganism, even, the production rate when these microorganisms are involved in the production of a particular compound, become necessary know the number and condition of this organism. In the actuality, there are a lot of tools to identify this condition, since kits of staining cells, until, the best microscopy technology. But the use of this tools could become inaccessible due the high cost and in the case of de staining kits had a limited test. Then emerge the necessity to search and implement news methodologies to help us to identify health and death cells in different inoculs or products. In this work, we proposed a new technology for the simultaneous observation of healthy and dead cells trough the staining samples with propidium iodide and acridine orange using an optic microscopy with incandescent light and ultraviolet light.

Healthy and dead cells, Microorganism staining, Acridine orange, Propidium iodide

Resumen

El uso de los microorganismos en un sin número de procesos e investigaciones, ha sido el estímulo fundamental para el desarrollo de nuevas tecnologías. En la búsqueda de garantizar el efecto que tendrán los microorganismos, su impacto o inclusive su taza de producción, cuando se emplean para la obtención de un compuesto en particular, es necesario conocer la cantidad y la condición de los organismos presentes. Actualmente existen un sinfín de herramientas para identificar esta condición, que van desde realizar la tinción de células, hasta, microscopios de última tecnología. Sin embargo, el uso de estos instrumentos puede volverse inaccesible debido a los altos costos de los equipos y en el caso de los kits se manejan para un número limitado de muestras. Por lo que surge la necesidad de buscar e implementar nuevas metodologías que ayuden a determinar células vivas y células muertas en diferentes inóculos o productos. En el presente trabajo se propone una metodología para la observación simultanea de células vivas y muertas, mediante la tinción de las muestras con ioduro de propidio y naranja de acridina y su observación en un microscopio óptico con luz incandescente y luz ultravioleta.

Células vivas y muertas, Tinción de microorganismos, Naranja de acridina, Ioduro de propidio

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* Correspondence to Author (email: naieli.herrera@utna.edu.mx)
† Researcher contributing as first author

Introduction

Currently, the potential that microorganisms represent is studied and exploited every day. The microorganisms are used as an alternative to obtain compounds used for the production of emerging medicines and even in the agricultural industry they are used as biocontrol agents for pests and as biofertilizers.

The wide range of application of microorganisms is due, in the case of bacteria, to the different bacterial interactions that they stimulate by combining different factors such as the activation of different metabolic pathways, production and secretion of signaling compounds, siderophores , antibiotics, plant protection against pathogens, plant growth promotion [Molina et at. 2019].

Microorganisms are currently the subject of many investigations, only in processes or analyzes of the environment, such as the detection of pathogenic bacteria in the environment [Singh et at. 1989], the identification of bacteria present in soils such as Pseudomonas spp. isolated from agricultural crop soils [Albarado et at. 2008],

An important element in the various investigations carried out in the aforementioned environment, whether with the objective of generating basic knowledge or its application, is the need to identify the viability of microorganisms, determine their location, their quantification, among others. The need to determine the viability of cells is important in many applications [Singh et at. 1989].

For the identification of microorganisms and even for the determination of the condition in which they are found, there are currently a wide range of methods to differentiate the viability of bacteria, to identify whether they are alive or not. These methods are based on differential staining, microcolony culture, microautoradiography or measurement of cellular respiration [Singh et at. 1989].

The staining methods have a wide range of application, they are not only used for the staining of microorganisms, but also, they have application in studies carried out with human cells for example for the monitoring of colon cancer cells in human cell cultures, during the incursion of new compounds to alter the condition of the cells [Kim et at. 2013].

They are also widely used for in vivo localization of subcellular differentiation [Gerhart et at. 2012]. There are different commercial kits that allow distinguishing between viable gram positive and gram negative bacteria. [Vázquez et at. 2010].

An alternative for bacterial observation is fluorescence microscopy. The fluorescence microscope is an optical microscope capable of illuminating the sample with a light whose wavelength may vary due to its passage through an excitation filter. This filter transmits exclusively the excitation light of the sample that has the selected wavelength. [Vázquez et at. 2010].

Propidium iodide is an impermeable fluorophore, which only crosses the membrane of dead cells. It interacts with nucleic acids, emitting red fluorescence (Emission: 617 nm) [Vázquez et at. 2010]. In contrast, acridine orange is a very permeable fluorochrome that acts as an intercalating agent, allows nucleotide staining, causing green fluorescence, and interacts with RNA causing orange fluorescence [Albarado et at. 2008; Kim et at. 2013, Vázquez et at. 2010]. Despite the progress and development of the methods currently used for the determination of cell viability, the development of methodologies or innovation in them, it is an indispensable tool for the development of new projects, it also represents an option to be able to perform an analysis in which less time is spent and even that represents a lower economic expense.

In the present work, a methodology for the simultaneous observation of living and dead cells is proposed, using an optical microscope with incandescent light and ultraviolet light, by staining the samples with the dyes of propidium iodide and acridine orange using a variation of the Kenneth and James methodology [Kenneth et at. 1985], simultaneously with the methodology of Hobbie et al. [Hobbie et at. 1977].

Objective

Develop a methodology that can simultaneously identify the presence of live cells and dead cells of Bacillus subtilis MZ in a modified starch matrix, using an optical microscope using incandescent light and ultraviolet light.

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Methodology

This project was developed in Cinvestav Unit Irapuato. The samples were observed under a microscope (BX50, Olympus, Japan) using 4X / 0.10 Plan (α - -) and 20X / 0.50 objectives, UPlan-FL (α - 0.17). The lighting methods were incandescent light whose source was halogen lamp (IOUSH, Japan) and ultraviolet light with mercury vapor lamp (OSRAM, Mexico) and excitation filter at 350 to 400 □m. Image acquisition was performed with a high sensitivity camera for Infinity3 fluorescence (Lumenera, Canada) synchronized through the Image Pro Premier 9.1 program (Media Cybernetics, USA).

The biological material or the samples analyzed were three different conditions of Bacillus subtilis MZ. The first condition was Bacillus subtilis MZ in a modified starch matrix, which is the objective of the investigation.

The other two conditions were implemented as a positive control of Bacillus subtilis MZ in the present methodology. Which consisted of samples of the same strain of the bacteria, but using vegetative cells and spores of our strain.

Staining of the samples was performed as described below:

1. Preparation of "stock" solutions

0.1% solution of acridine orange in sterile distilled water.

2% solution of propidium iodide in saline phosphate regulator as mentioned by Kenneth and James [1985].

2. Staining of Bacillus subtilis MZ in the modified starch matrix

1.- A solution called "staining solution" was prepared with the "stock" solutions in a 1: 1 ratio. This solution was prepared at the time each of the observations was made.

2.- To a sample of our study subject, mounted on a slide, 10 µl of the staining solution was added.

3.- Samples were observed under a microscope (BX50, Olympus) at two wavelengths, the first of 480-510 ηm m and the second of 530-550ηm.

4.- A splicing of the photographs taken for the observations at both wavelengths was made, to appreciate live cells and dead cells in the same image and see if they co-locate.

3. Staining of Bacillus subtilis MZ "Positive Control 1"

As a positive control, Bacillus subtilis MZ cells (in the absence of the modified starch matrix) were observed, to make the observations the cells were obtained as follows:

1.- Bacillus subtilis MZ was grown in potato infusion, incubated at a temperature of 23 ° C for 24 hours.

2.- To eliminate background noise in microscopic observations caused by the culture medium, the cells were separated by centrifugation at 10,000 rpm for a time of 5 minutes at 4 ° C.

3.- Two rinses were made with sterile distilled water, under the same centrifugation conditions. The supernatant was discarded and the pellet was resuspended in sterile distilled water.

4.- To harvest the cells, it was centrifuged again under the same operating conditions. The pellet was resuspended in 200 µl of the staining solution, allowed to stand for 3 minutes. For staining, 200 µl of the staining solution was used for every 100 µl of culture sample.

5.- The cells were separated from the dye by centrifugation, in the same way as in step 1. The pellet was resuspended in 200 µl of sterile distilled water.

6.- A 7 µl aliquot of the sample was mounted on a slide, observations were made with the microscope at two wavelengths, 480-510 □m and 530-550ηm m.

7.- A splicing of the photographs taken for observations at both wavelengths was made to see live cells and dead cells in the same image and see if they co-locate.

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4. Staining of Bacillus subtilis MZ spores "Positive control 2"

As a second positive control, Bacillus subtilis MZ spores were observed, the samples were obtained in the manner described in the previous paragraph, unlike the culture of B. subtilis MZ was incubated for 4 days to allow the greatest number of spores.

For each sample three stains were made of which, a total of 5 fields were observed at both mentioned wavelengths.

Results and Discussion

The samples of Bacillus subtilis MZ stained with acridine orange were observed simultaneously with propidium iodide, using the methodology previously described. All photographs were taken at the different lengths specified, using the Image Pro Premier 9.1 program.

Our main subject of study was Bacillus subtilis MZ in a modified starch matrix. It was possible to observe and identify clearly and quickly the living cells in green, the dead red cells. The analysis of the splicing of the two conditions was carried out to verify that they do not co-locate and to confirm that the selectivity of the dyes used for staining (Figure 1).

Green fluorescence was observed in living cells, because the orange acridine dye binds to the bacterium RNA by electrostatic interactions. Likewise, the dead cells were observed red, by the passage of the propidium iodide through damaged membranes since this is interspersed with DNA chains, in addition, this dye is excluded from intact membranes, which makes it a dye effective to identify non-viable cells. The interaction of the bacterial genetic material, DNA, RNA with the two dyes simultaneously causes fluorescence in the different colors [Albarado et at. 2008; Kenneth et at. 1985].

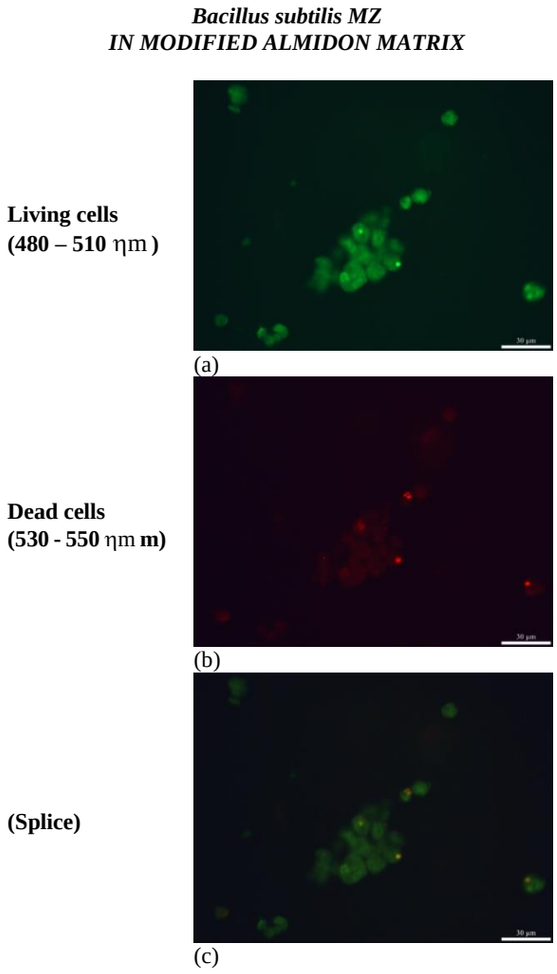

Figure 1 Bacillus subtilis MZ in a modified starch matrix. Photographs of the results obtained by simultaneous staining of acridine orange with propidium iodide

The proposed methodology based on simultaneous staining with acridine orange and propidium iodide, in this work is presented as a tool for the qualitative analysis of the viability of bacteria. This methodology, with the necessary complementary equipment, can be used to determine the viability of the cells quantitatively, by measuring the intensity of the emitted fluorescence [Hussain et at. 2019].

The propidium iodide also allows staining of damaged cells [Kirchhoff et at. 2017]. A culture of Bacillus subtilis MZ in a vegetative state was established as the first positive control (Figure 2).

For the first positive control, the presence of living cells was corroborated (Figure 2a), as well as the presence of dead cells (Figure 2b), splicing of the images was performed to analyze the co-location of the bacteria (Figure 2c).

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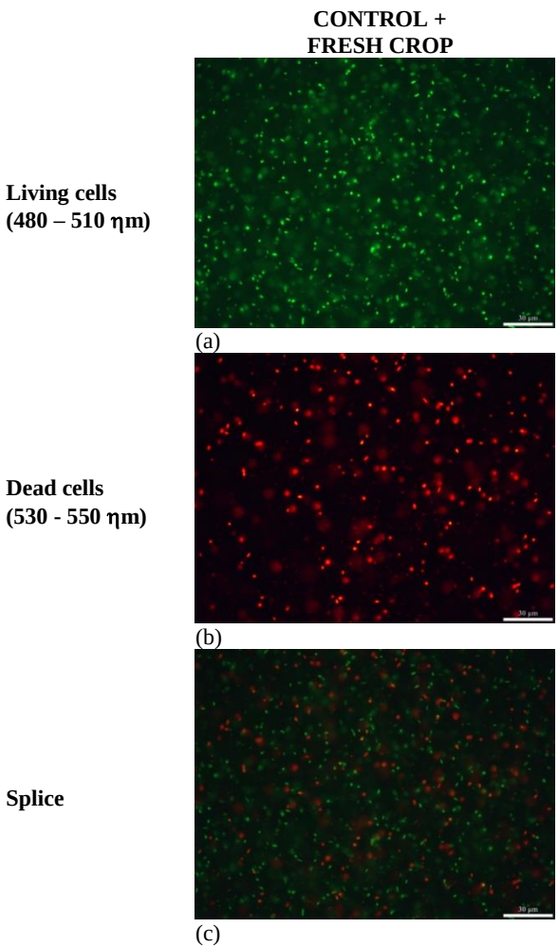
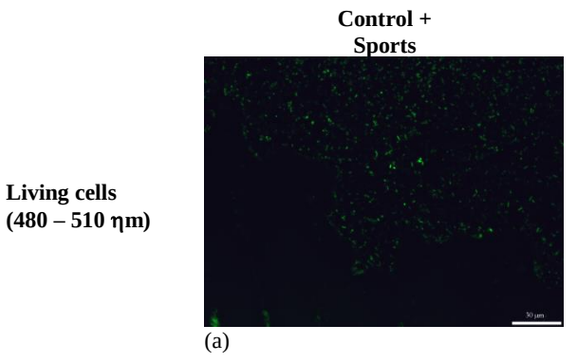


Figure 2 Vegetative cells of Bacillus subtilis MZ. Photographs of the results obtained by simultaneous staining of acridine orange with propidium iodide

A culture of Bacillus subtilis MZ spores was evaluated as a second positive control (Figure 3). The spores were observed in the length of living cells, due to their interaction with the dye (Figure 3a), as well as the presence of dead cells in the sample (Figure 3b). The same co-location analysis used for the other samples was performed (Figure 3c). These results indicate that part of the cells that are stained with propidium iodide as dead cells may be spores of B. subtilis MZ.



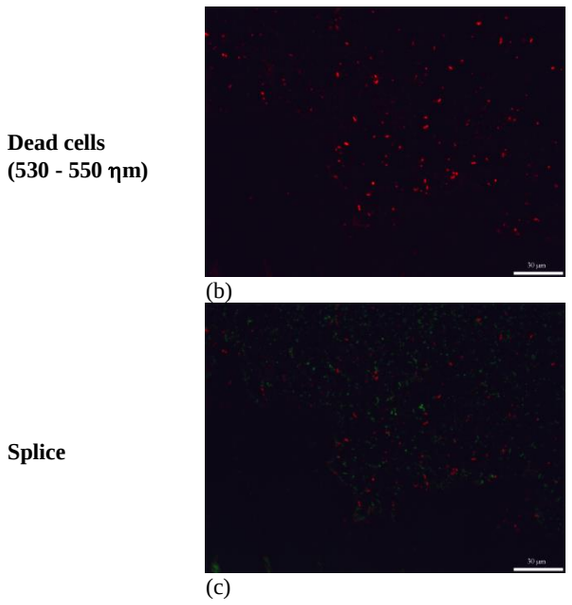
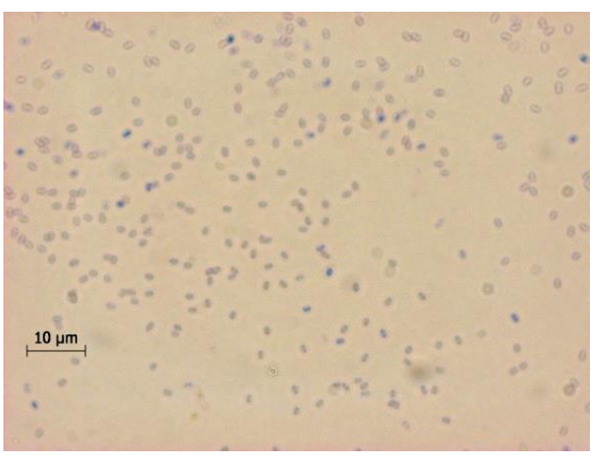


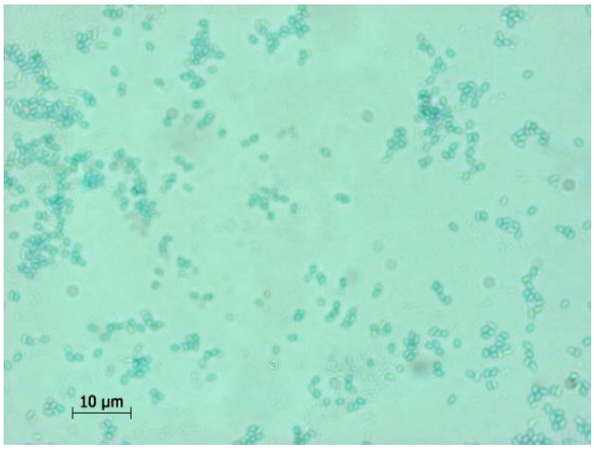
Figure 3 Bacillus subtilis MZ spores. Photographs of the results obtained by simultaneous staining of acridine orange with propidium iodide

The second positive control was also analyzed by Gram staining (Figure 4a) and malachite-safranine green staining (Figure 4b). The green Bacillus subtilis Mz spores were successfully identified because the malachite green penetrates the spore membrane, the use of safranine is to obtain a contrast with the cells in a vegetative state.

The spores present multiple aspects of interest, represent an important process of cell differentiation or morphogenesis perfectly regulated and controlled by the bacteria, [Vázquez et at. 2010]. Staining with malachite-safranine green allows the bacterial endospora to be revealed, a form of resistance produced by some genera of gram-positive bacteria. [Vázquez et at. 2010].



(a)



(b)

Figure 4 Photograph of (a) Gram staining, (b) staining with malachite green- safranine both Bacillus subtilis MZ

Conclusions

The proposed methodology allowed to observe simultaneously live cells and spores of the bacterium B. subtilis MZ. It is an option in which less time and economic resources are invested to identify the viability and the location of microorganisms. As mentioned by Ortega et al. [Ortega et at. 2010], one of the advantages of the tests, is not only its ease and cost, but also that the information it provides is very useful for the analyzed factor.

Albarado et al. [Albarado et at. 2008], also conclude that modified fluorescence differential coloring methods can be used as a complementary technique in clarifying the molecular mechanisms involved in the cell cycle. The use of the present methodology coincides with that proposed by Vázquez et al. [Vázquez et at. 2010] who express that for the observation of bacteria various general fluorochromes can be used both in crops, as in samples from natural environments (soil, water, interior of living beings) or artificial (sludge digesters, composting piles, biological reactors , etc.). [Vázquez et at. 2010]. Hussain and food collaborators [Hussain et at. 2019] have used this type of dyes to determine chemical and microbiological profiles in food samples.

The scope of the work developed could be unlimited, because the methodology developed can be used for any bacterium with the certainty of the results obtained in the present study.

Only the existing interaction with the vehicle in which the bacteria to be evaluated would have to be evaluated, in our case the starch matrix did not represent an impediment to the observation of the condition of Bacillus subtilis MZ.

An important factor that must be considered for the use of the established methodology is the solubility of the dyes used with the medium in which the bacterium to be analyzed is found, in the case of the present study the modified starch matrix is soluble in the work solutions used.

As a perspective, we propose the analysis that has the effect of the amount of RNA and DNA present in the cell according to the phase of its cell cycle, in the staining of the bacteria [Albarado et at. 2008]. It is also proposed to evaluate the membrane potential, because it has been reported that it is a parameter that affects cell staining [Kirchhoff et at. 2017].

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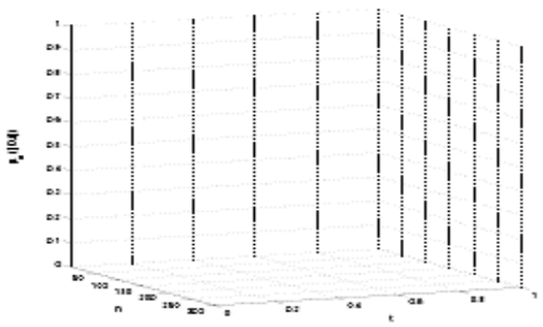

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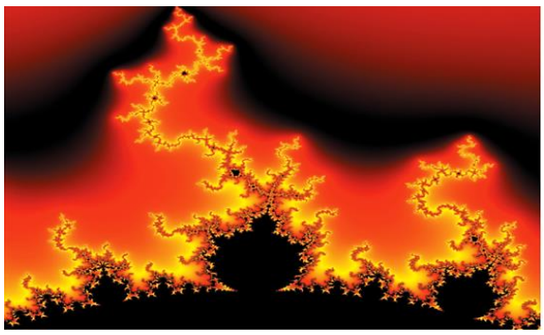

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