BIOFERTILIZATION OF FORAGE SORGHUM TO INCREASE POLYPHENOL LEVELS FOR A POSSIBLE ANTI-METHANOGENIC EFFECT

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SUMMARY

Methane gas is one of the main contributors to global warming, as it traps more heat than carbon dioxide in the atmosphere; livestock produce a significant percentage of this gas, so one viable alternative is to reduce methanogenesis in the rumen by providing a diet rich in polyphenols. In the present study, the increase in polyphenolic compounds in forage sorghum was evaluated, since this crop is commonly used as cattle feed. Four treatments were applied: a mixture of Bacillus *sp.,* Azospirillum brasilense*,* Trichoderma*, a combination of fungi and bacteria, and a control group. These treatments were tested on seven varieties of forage sorghum grown in Mexico (Cañaveral, Sureño, MXSE45, Tehua, Cañero, MXSE2, and MXSE19). On days 20 and 56 after planting, morphoagronomic parameters were measured (plant height, stem diameter, and photosynthetic activity), as well as the concentrations of phenols, flavonoids, and tannins. Variety MXSE19 showed the highest values for height, plant weight, and phenol concentration, with the* Trichoderma *treatment being the most effective variety–microorganism combination. The Tehua and Cañero varieties exhibited the lowest values across all treatments. The use of biofertilizers in forage represents a potential strategy to increase the concentration of polyphenols, which have anti-methanogenic activity.*

Introduction

Methane (CH_4), produced by natural and anthropogenic sources, has increased in atmospheric concentration from 1,650 to 1,932.23 parts per billion (ppb) over the last 35 years (Džermeikaitė *et al*., 2024; Lan and Dlugokencky, 2024). Livestock farming systems are major emitters of methane; the main greenhouse gases they produce are CH_4 (43%), nitrous oxide (N_2O, 29%) and carbon dioxide (CO_2, 27%). Ruminants contribute approximately 20% of global CH_4 emissions; this gas is generated primarily through the anaerobic decomposition of organic compounds in forages by bacteria (Džermeikaitė *et al*., 2024; Merino *et al*., 2011). During this process, archaea use dihydrogen and carbon dioxide to synthesize CH_4 (Merino *et al*., 2011).

KEYWORDS / Anti-Methanogenesis / Forage / Global Warming / Greenhouse Effect / Livestock / Polyphenols / Sorghum /

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There is growing scientific interest in controlling ruminal methane production due to its environmental impact and the associated energy losses (Haque, 2018). Several CH_4 mitigation strategies are currently under investigation, including modification of diet composition, selection of low-emission animals, intensification of production systems, use of secondary plant compounds, chemical inhibitors, immunization, defaunation, reductive acetogenesis, and bacteriophages (Ungerfeld *et al*., 2018). A natural mitigation alternative that has gained relevance in animal nutrition is the use of secondary plant metabolites. Several studies have demonstrated the capacity of certain compounds to reduce methanogenesis through different mechanisms of action (Goel and Makkar, 2012; Kim *et al*., 2015; Moscoso *et al*., 2017; Vélez-Terranova *et al*., 2014). Additionally, research has been conducted on the use of microorganisms as biofertilizers, which may enhance the production of plant metabolites (Fasusi *et al*., 2018). Furthermore, the use of microorganisms as fertilizers is considered an eco-friendly alternative to chemical fertilizers, promoting plant growth and improving soil fertility (Kour *et al*., 2020). The aim of the present study was to evaluate the potential of biofertilization to increase the production of polyphenolic compounds in forage sorghum, given that these secondary metabolites have potential anti-methanogenic activity.

Materials and Methods

Four treatments were evaluated: the first consisted of a mixture (1:1.5:1.5) of *Bacillus atrophaeus* and *Bacillus velezensis* to achieve a final concentration of 1×10^9CFU/ml, combined with *Azospirillum brasilense* (BA); the second treatment was *Azospirillum brasilense* (AZ); the third, *Trichoderma* spp. (TRI); and the fourth, a combination of *Azospirillum brasilense*, *Glomus* spp., *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Bacillus mucilaginosus* (BH), containing 50 million CFU/g and at least 33 endomycorrhizal fungal spores per gram. Finally, a control group (T) was included. These treatments were applied to seven forage sorghum varieties: Cañaveral (CAÑAV), MXSE45 (MX45), Sureño (SUR), Tehua (TEH), Cañero (CAÑ), MXSE2 (MX2), and MXSE19 (MX19). The plant material was provided by the National Forestry, Agriculture and Livestock Research Institute (INIFAP, Rio Bravo Campus, Tamaulipas, Mexico) and analyzed using a factorial design to evaluate the effect of biofertilizers (first factor) and sorghum varieties (second factor) on morphoagronomic traits and polyphenol production.

The experiment was conducted in the greenhouse of the Genomic Biotechnology Center at the National Polytechnic Institute (Reynosa, Tamaulipas, Mexico). One hundred seeds of each variety were sown in two pots filled with peat moss. Eight days after planting, germination percentage was determined by calculating the ratio of germinated plants to the total number of seeds sown. On days 20 and 56 after planting, twenty plants per treatment were measured for the following morphoagronomic parameters: plant height (cm), measured with a wooden ruler from the base to the highest point; stem diameter (mm), measured with a calibrated digital caliper; and photosynthetic activity (SPAD units), determined with a SPAD-502 PLUS chlorophyll meter (KONICA MINOLTA, INC.). Three additional plants per treatment were collected to evaluate metabolite concentrations on the same dates as the agronomic measurements.

For metabolite analysis, leaves were harvested and dried in an oven (Felisa Model FE-291) at 45°C for 24h. Then, 0.25g of dry tissue was weighed, mixed with 1ml of absolute ethanol, and left to stand for 30 min. The mixture was centrifuged at 5000 rpm for 10 min, and the leaf extract was collected and stored at −5°C until analysis.

The concentration of total phenols was determined using the Folin–Ciocalteu method (Dewanto *et al*., 2002; Singleton *et al*., 1999; Singleton and Rossi, 1965), and absorbance was measured in a UV–Vis spectrophotometer (UV-1800 Shimadzu) at 760nm. Tannin concentration was determined using the method of Broadhurst and Jones (1978), with absorbance measured at 500nm. Flavonoid concentration was assessed according to the method of Heimler *et al*. (2005), with absorbance measured at 510nm. All analyses were performed in triplicate using three dilutions (1:10, 1:100, and 1:1000). Phenol content was expressed as gallic acid equivalents (mg GAE/g dry matter), while flavonoid and tannin contents were expressed as catechin equivalents (mg CE/g dry matter). Data were analyzed using a factorial design, considering treatment and variety as fixed factors and their interaction; mean comparisons were performed using Tukey's test in RStudio (version 4.0.4).

Results

The germination percentage was evaluated eight days after sowing. Significant statistical differences were found among varieties ($p= 0.007$), but not among treatments ($p= 0.87$) nor for the treatment-by-variety interaction ($p= 0.88$). Four of the seven varieties achieved germination percentages above 90%, while only the MX19 variety showed germination rates below 70%.

Significant differences were also observed in plant height, both for the main factors (treatment, $p= 0.00774$; variety, $p= 7.849e-12$) and for the treatment-by-variety interaction ($p= 8.881e-07$). The *Trichoderma* treatment showed the highest mean plant height (38.64cm), whereas the control group recorded the lowest average (34.1cm). The Tehua variety reached the greatest height (40.21cm), while the Cañero variety showed the lowest value (29cm). Figure 1 illustrates the full treatment–variety interactions for plant height.

The MX19 variety treated with BH recorded the greatest plant height (46.5cm). Three of the five tallest mean plant heights corresponded to varieties treated with *Trichoderma* spp. Conversely, the Cañero (CAÑ) variety without treatment showed the lowest value (26.6cm), and three of the five shortest averages were also from this variety.

Statistical differences were found in stem diameter for both main factors (treatment = 2.54e-09; variety, $p= 0.00032$) and for the treatment-by-variety interaction ($p= 0.001131$). Figure 2 presents the complete treatment–variety interactions for stem diameter. Three of the five largest stem diameters were observed in plants treated with *Trichoderma* spp.; the CAÑAV variety reached the greatest value (2.65mm). Notably, the CAÑ variety, under two different treatments (TRI and AZ), appeared among the five best-performing combinations. On the other hand, the control group and the BA treatment showed the lowest average stem diameters. The SUR variety with the BA treatment exhibited the smallest stem diameter (1.45mm).

Finally, photosynthetic activity results are shown in Figures 3a and 3b. No significant interaction effect was detected between treatment and variety ($p= 0.393$), but significant differences were found for the main factors (treatment, $p= 1.12e-07$; variety, $p= 9.40e-16$). Regarding treatments, *Trichoderma* spp. recorded the highest photosynthetic activity (31.26 SPAD units), followed by AZ (30.89 SPAD units), while the control group showed the lowest mean value (27.84 SPAD units). Regarding varieties, MX19, MX45, MX2, and Tehua reached the highest averages (32, 31.6, 30.9, and 31.2 SPAD units, respectively), while

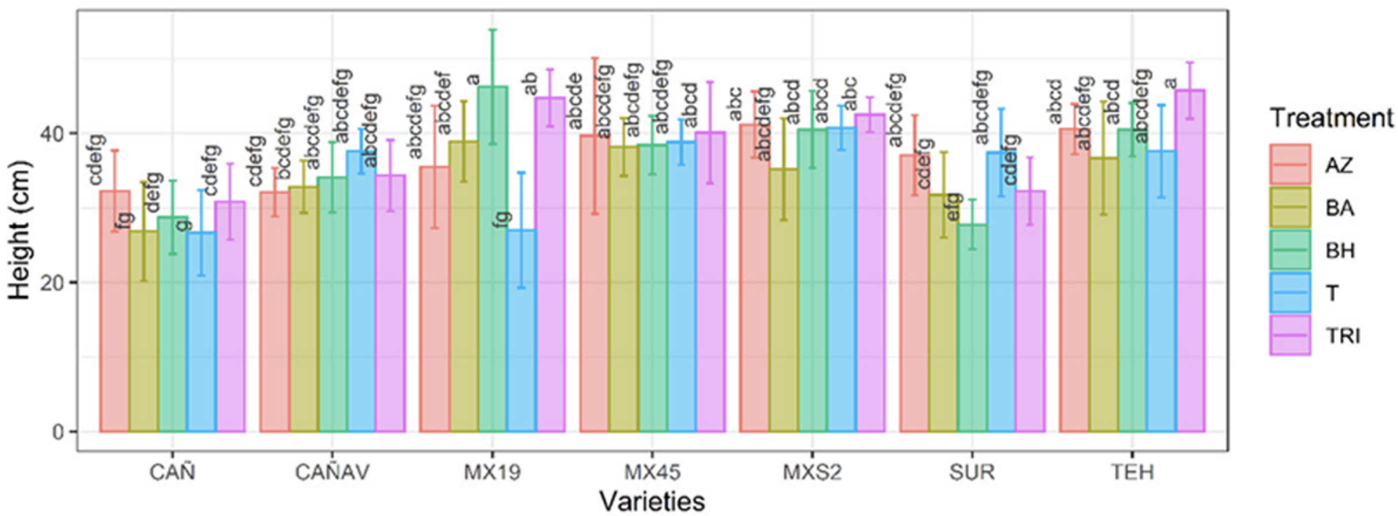

Figura 1. Plant height of seven varieties of forage sorghum subjected to different biofertilizers BA: Mix of *Bacillus atrophaeus*, *Bacillus velezensis* and *Azospirillum brasilense*; AZ: *Azospirillum brasilense*; TRI: *Trichoderma* spp.; BH: combination of *Azospirillum brasilense*, *Glomus* spp, *Trichoderma* spp, *Bacillus subtilis*, *Pseudomonas flourescens*, *Bacillus mucilaginosus*; T: control group.

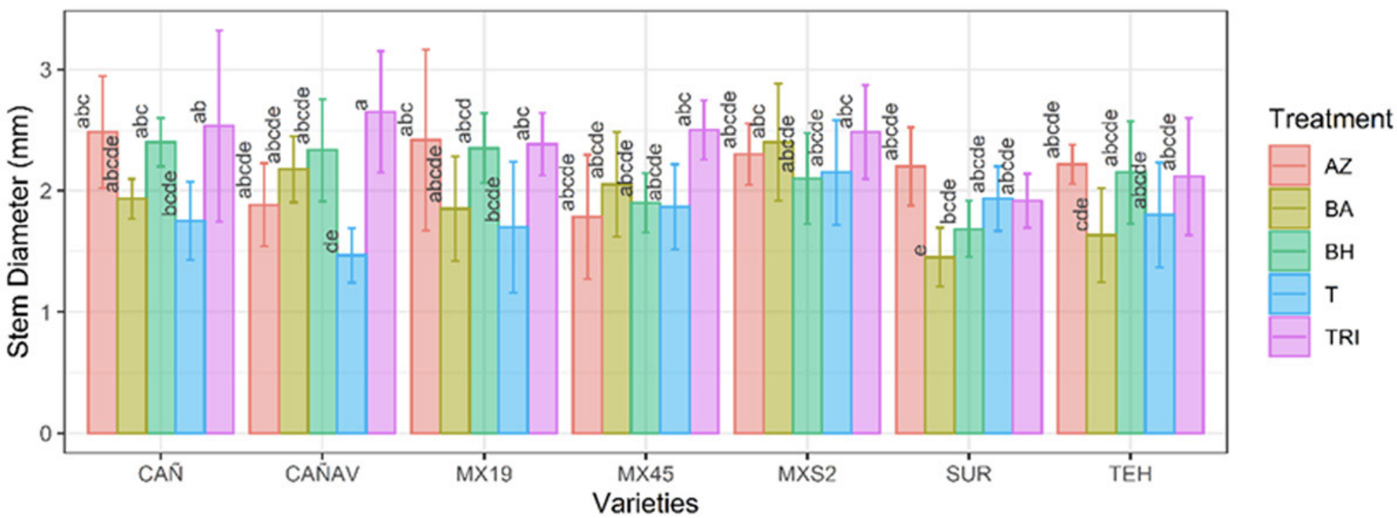

Figura 2. Steam diameter of seven varieties of forage sorghum subjected to different biofertilizers.

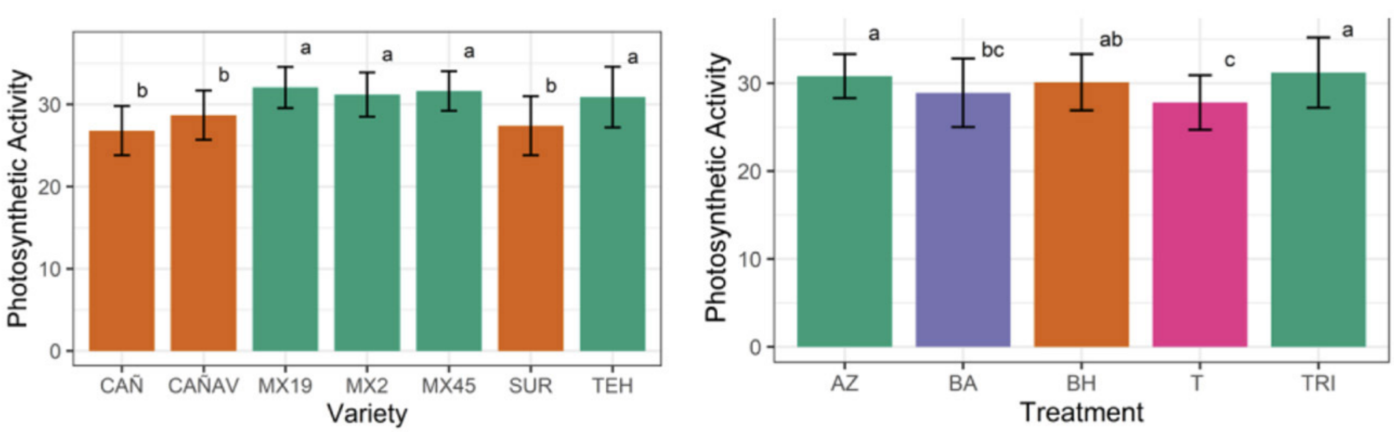

Figura 3. Average of photosynthetic activity for varieties (a) and treatments of forage sorghum subjected to different treatments (b).

CAÑAV, CAÑ, and SUR exhibited the lowest averages.

The determination of secondary metabolites —phenols, tannins, and flavonoids— was performed using spectrophotometric methods. Significant statistical differences were detected for both the main factors and their interaction ($p < 2e\text{-}16$). Tables I and II present the secondary metabolite contents at both sampling dates, organized by treatment and variety. Additionally, the treatment–variety interaction was analyzed.

Overall, plants treated with biofertilizers exhibited higher metabolite concentrations compared with the control group. Phenol levels increased consistently on day 56 compared with day 20, while tannins showed slightly higher values on day 56 than on day 20. Sorghum treated with *Trichoderma* spp. showed the highest phenol concentrations across all varieties. Phenolic content increased on the second sampling date; among the seven varieties studied, MX19 displayed the highest concentrations of phenols and flavonoids in both sampling periods ($p < 2e\text{-}16$) (Figures 4 and 5).

In general, *Trichoderma* treatment resulted in the highest phenol concentrations in the CAÑ, MX19, and MX2 varieties (26.5, 26.3, and 25.1GAE/g, respectively). The control treatment combined with the Tehua variety produced the lowest value (0.06 mg GAE/g). Figure 5 illustrates the full treatment–variety interactions for phenolic content on day 56. Similar to the first sampling, varieties treated with Trichoderma spp. recorded the highest phenol levels; three of the five top-performing varieties (TEH, MX45, and MX19) showed averages above 40mg GAE/g. However, the MX19 variety achieved the highest mean value when treated with Azospirillum brasilense (59.09mg GAE/g), while the MX2–AZ combination recorded the lowest average (14.33mg GAE/g).

For flavonoids, detectable concentrations were found only at the 20-day sampling (Figure 6); by day 56, values were negligible. The treatment–variety interaction showed significant differences ($p < 2e\text{-}16$). Regarding tannins at the first sampling (20 days), the BA treatment in TEH and MX45 varieties showed the highest averages (52.4 and 10.8mg CE/g, respectively). It was also observed that 27 out of 35 treatment–variety combinations recorded no measurable tannin content (Figure 7). The CAÑ variety treated with the BH combination (bacteria and fungi) recorded the highest mean value (25.0mg CE/g). Five varieties treated with *Azospirillum* spp. were among the highest averages. By day 56, twenty treatment–variety combinations showed no detectable tannin content (Figure 8).

Discussion

Climate change is transforming ecosystems worldwide. To keep the increase in global temperature below 2°C and avoid dangerous climate impacts, substantial reductions in greenhouse gas (GHG) emissions are urgently required (Gerber *et al*., 2003). Livestock production is a major global source of GHGs. Depending on the methodology and emission categories considered, international

TABLE I
TOTAL PHENOL, TANNIN AND POLYPHENOL CONTENT OF SORGHUM PLANTS TREATED WITH DIFFERENT BIOFERTILIZERS, AT 20 AND 56 DAYS AFTER SOWING

Time (days)	Treatments	Total phenols (mg GAE/g)	Flavonoids (mg CE/g)	Tannins (mg CE/g)
20	Control	10.00d	6.75b	0.0c
	BA	10.4cd	2.9e	10.4a
	TRI	17.2a	7.50a	0.10c
	AZ	11.1c	5.2c	4.2b
	BH	13.4b	4.1d	0.1c
56	Control	22.8d	0.0	0.7 d
	BH	23.3d	0.0	5.6b
	TRI	32.9a	0.0	0.0d
	AZ	29.7b	0.0	13.5a
	BA	24.8c	0.0	2.9c

Values with different letters have a statistically significant difference, according to Tukey's test ($p < 0.05$). BA: Mix of *Bacillus atrophaeus*, *Bacillus velezensis* and *Azospirillum brasilense*; AZ: *Azospirillum brasilense*, TRI: *Trichoderma* spp.; BH: combination of *Azospirillum brasilense*, *Glomus* spp, *Trichoderma* spp, *Bacillus subtilis*, *Pseudomonas flourescens*, *Bacillus mucilaginosus*); T: control group.

TABLE II
TOTAL PHENOL, TANNIN AND POLYPHENOL CONTENT OF SORGHUM VARIETIES TREATED WITH DIFFERENT BIOFERTILIZERS, AT 20 AND 56 DAYS AFTER SOWING

Time (days)	Variety	Total phenols (mg GAE/g)	Flavonoids (mg CE/g)	Tannins (mg CE/g)
20	Mxse19	15.0a	6.4a	1.3d
	Cañero	14.9a	5.7b	0.0f
	Mxse2	13.3b	4.7cd	1.7c
	Tehua	7.1d	4.2d	10.5a
	Mxse45	13.8b	5.2bc	2.2b
	Cañaveral	8.9c	6.5a	0.1f
	Sureño	13.8b	4.3d	0.5e
56	Mxse19	33.7a	0.0	4.6c
	Cañaveral	21.5e	0.0	5.5b
	Sureño	25.9c	0.0	0.0d
	Tehua	31.1b	0.0	4.2c
	Cañero	23.9d	0.0c	13.0a
	Mxse45	25.7c	0.0	0.8d
	Mxse2	25.2c	0.02b	3.8c

Values with different letters have a statistically significant difference, according to Tukey's test ($p < 0.05$).

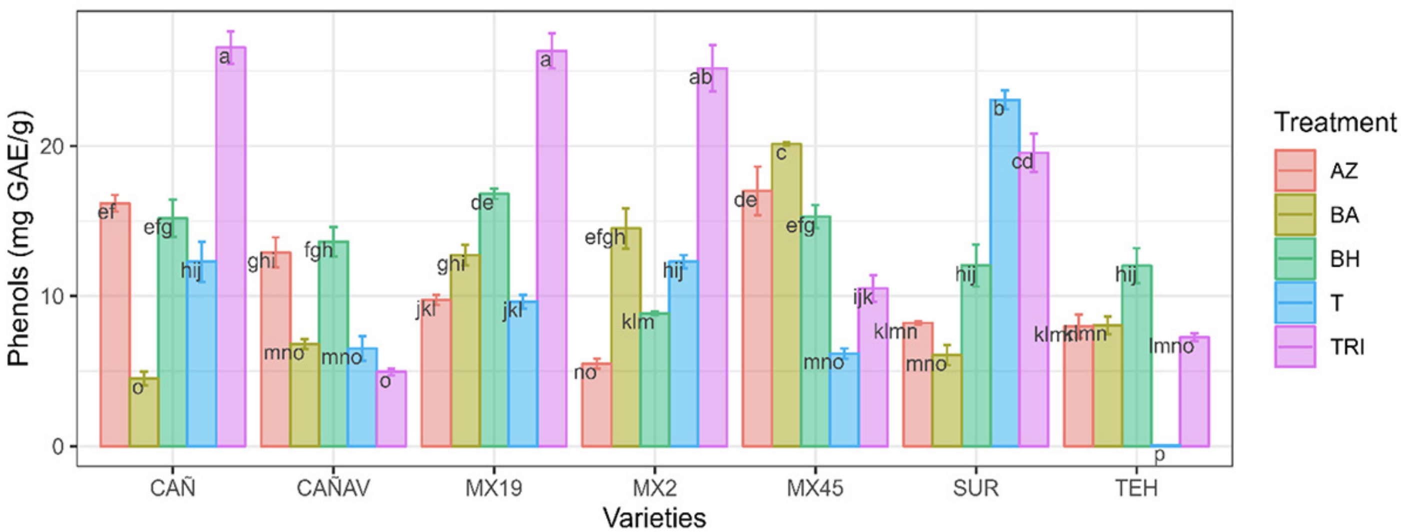

Figura 4. Treatment by variety interaction of forage sorghum for the phenol metabolite on day 20 of the treatment.

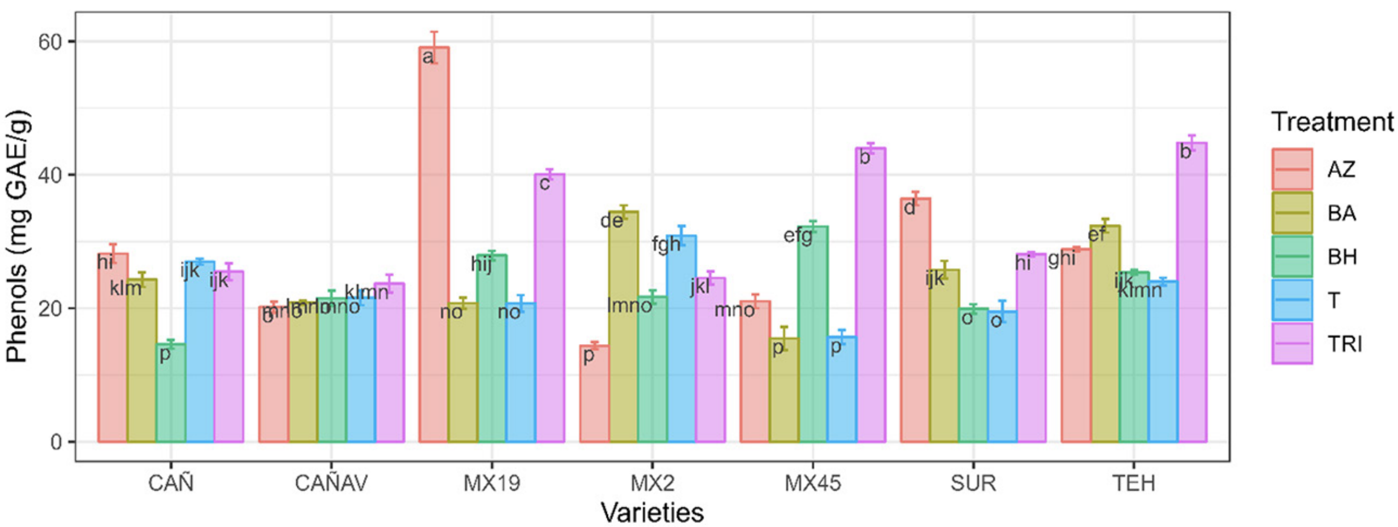

Figura 5. Treatment by variety interaction of forage sorghum for the phenol metabolite on day 56 of the treatment.

organizations (IPCC, FAO, EPA, among others) have estimated that cattle contribute between 7% and 18% of total anthropogenic GHG emissions worldwide (Hristov *et al*., 2013). Methane (CH_4) is generated primarily by the anaerobic decomposition of organic compounds in the bovine digestive tract and is released into the atmosphere mainly through enteric fermentation (burping).

Hristov *et al*. (2013) identified several strategies to mitigate this effect, including archaeal inhibitors such as bromochloromethane, electron acceptors such as nitrates, ionophores, plant-derived metabolites, exogenous enzymes, defaunation, manipulation of ruminal microbiota, and improved grazing and feeding management. Within the latter category, silvopastoral systems —based on integrating trees or shrubs, legumes, and grasses into livestock feeding— have been studied as a sustainable option (Gutiérrez-Bermúdez and Mendieta-Araica, 2022). Silvopastoral systems aim to maximize resource-use efficiency, evaluate competitive interactions in perennial systems, and promote structural and functional diversity to conserve resources, enhance animal production, improve soil fertility, and increase forage quality (Kour *et al*., 2020).

Mathematical models suggest that progressively eliminating the main global sources of CH_4 and N_2O while allowing biomass to recover could achieve sustained reductions in CO_2-equivalent emissions over the next 30 years (Eisen and Brown, 2022). The present study evaluated the effect of biofertilizers on the production of secondary metabolites with anti-methanogenic potential and on the agronomic performance of forage sorghum; the latter aspect, if improved, could provide economic benefits for

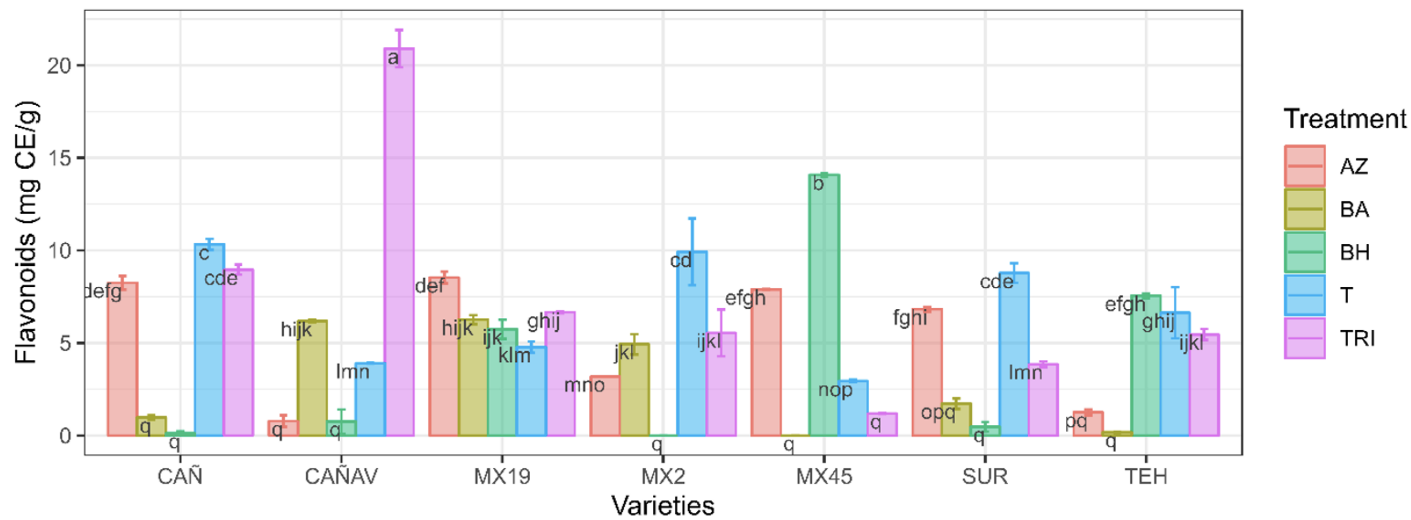

Figura 6. Treatment by variety interaction of forage sorghum for the metabolite flavonoids on day 20 of the treatment.

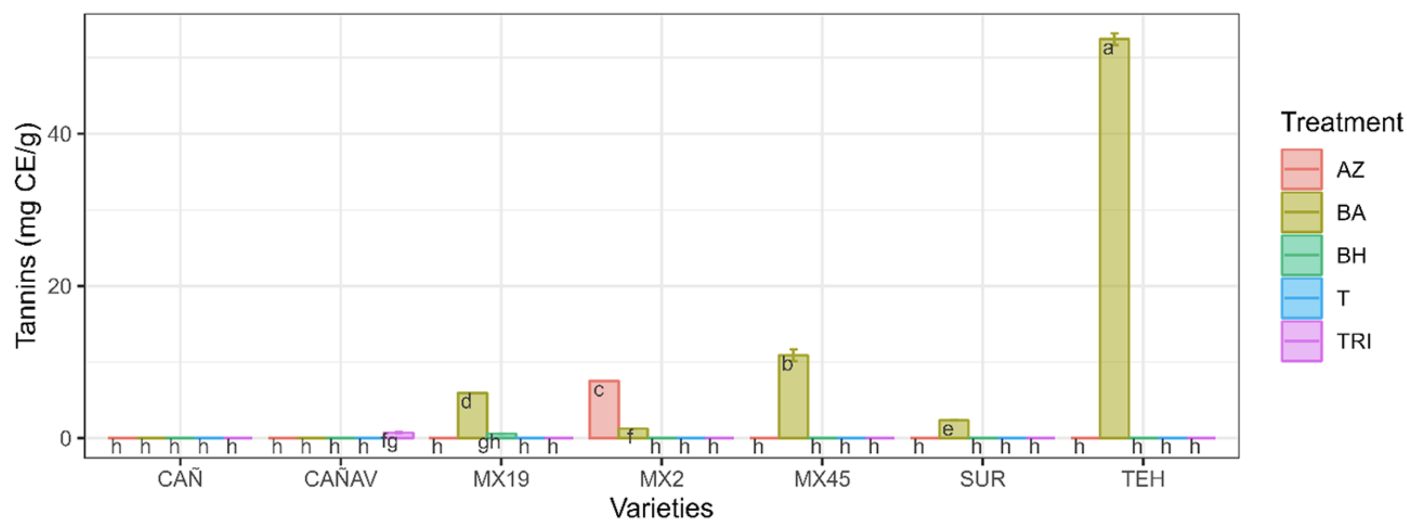

Figura 7. Treatment by variety interaction of forage sorghum for the metabolite tannins on day 20 of the treatment.

livestock producers and reduce the need for chemical fertilizers.

In general, the treatments applied in this study —based on bacteria, fungi, or their combinations— resulted in greater plant height, stem diameter, and photosynthetic activity in the different sorghum varieties compared with the control group. The treatment with *Trichoderma* spp. consistently produced higher values than the other treatments; TEH, MX19, and MX2 exhibited the greatest plant heights. Three of the top-performing varieties in terms of stem diameter were also treated with *Trichoderma* spp. (Figure 2). Similarly, this treatment showed the highest photosynthetic activity (31.26 SPAD units).

Additionally, the varieties MX19, Tehua, and MX45 recorded higher values in several agronomic parameters under different treatments. The physiological basis of these effects has been widely investigated, with rhizobacteria and cyanobacteria providing environmentally sustainable strategies to enhance crop growth and health (Singh *et al*., 2011). The mechanisms of action of these microorganisms include the production of phytohormones and siderophores, phosphate solubilization, and induction of plant resistance to biotic and abiotic stress (Santos *et al*., 2019). Kour *et al*. (2021) highlighted the ability of several genera —including *Arthrobacter, Bacillus*, *Burkholderia*, *Natrinema*, *Pseudomonas*, *Rhizobium* and *Serratia*— to solubilize phosphate, making it available to plants.

Among plant-growth-promoting rhizobacteria, *Azospirillum* spp. are widely studied; at least 21 species are recognized, with *Azospirillum brasilense* being the most extensively investigated. Its mechanism of action is primarily associated with the production of phytohormones, particularly indole-3-acetic acid (IAA) (Licea-Herrera *et al*., 2020). Similarly, the fungus *Trichoderma* spp. is

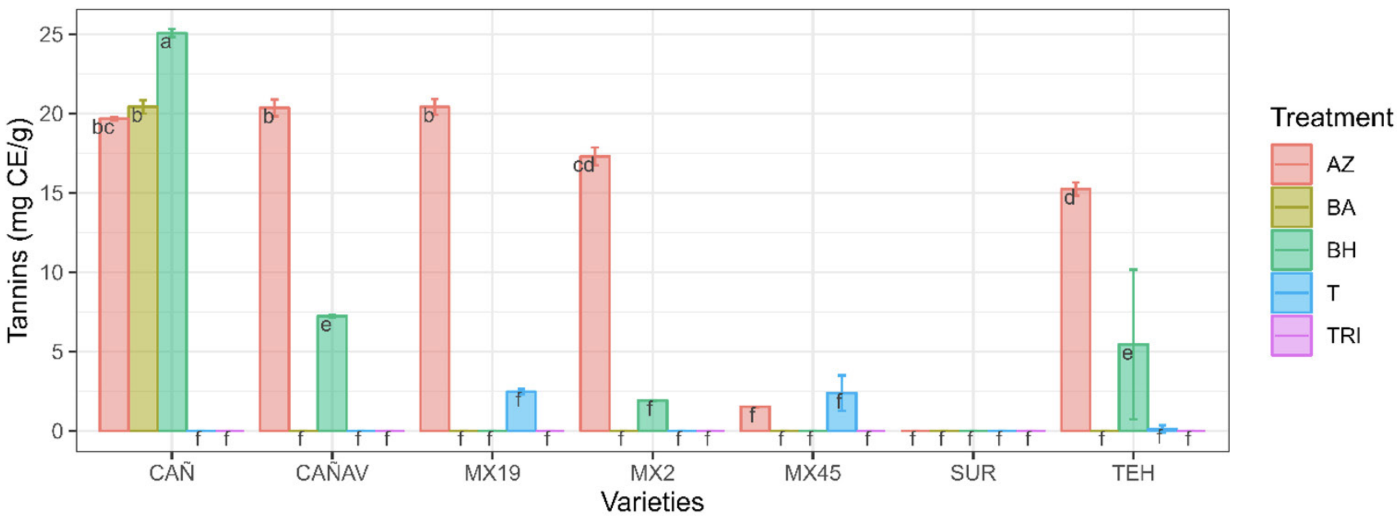

Figura 8. Treatment by variety interaction of forage sorghum for the metabolite tannins on day 56 of the treatment.

known to produce IAA and to degrade the seed episperm, participate in respiratory processes during germination, and act as an antagonist of several phytopathogenic fungi, making it a key microorganism for biofertilization (Mendoza *et al*., 2011).

Successful use of different biofertilizers in forage production has been previously documented for rainfed maize, the grass *Brachiaria brizantha* (Hernández-Sánchez *et al*., 2018), the legume *Lotus corniculatus* in high tropical areas (Santacoloma-Varón *et al*., 2017), and hydroponically grown maize (Rangel *et al*., 2014).

Regarding the role of metabolites in methane (CH_4) mitigation, the first review of phenolic compounds in mammals by Singleton (1969) highlighted their potential toxic effects. Subsequently, several plant secondary metabolites have been shown to alter and suppress ruminal microbial populations. Lowry *et al*. (1996) reported that their effects range from occasional acute toxicity of hydrolyzable tannins to complex interactions between tannins and proteins. The diverse impacts of plant phenols on nutrient flow are likely explained by the balance between their adverse effects on some microorganisms and the rate at which they are degraded or inactivated by others. Manipulating ruminal microbial metabolism may therefore improve animal performance.

In this study, among the three metabolites analyzed, phenols were found in the highest concentrations and even increased at the second sampling date. Fertilization with *Trichoderma* spp. resulted in significantly higher phenol values than all other treatments on both day 20 and day 56, with the latter showing the maximum concentration (32.94mg GAE/g). The MX19 variety also recorded the highest phenol concentrations on both sampling dates (Figures 4 and 5), differing significantly from the other treatments.

Plants with high polyphenol content are considered a potential strategy to reduce ruminal methane (CH_4) emissions, although this may occur at the expense of nutrient utilization. However, non-additive effects may arise when phenol-rich plants are combined with nutrient-rich, highly digestible feeds (Jayanegara *et al*., 2013). Proper pasture management can provide young, highly digestible forage. Gurbuz (2009) reported reductions in total gas, methane, and carbon dioxide production in seven forage legume species, attributed to their condensed tannin content. This effect may result from enhanced fiber degradation as well as residual inhibition of methanogens.

Multiple factors influence methanogenesis, including the type of tannins and plant species. A higher number of hydroxyl groups is associated with greater methane reduction potential. Condensed tannins reduce methane primarily by limiting fiber digestion (an indirect effect), whereas hydrolyzable tannins appear to act directly by inhibiting methanogen growth and/or hydrogen availability (Goel and Makkar, 2012). Increasing concentrations of hydrolyzable tannins tested in goat rumen fluid reduced methanogenesis (mL/250mg DM) without affecting other fermentation parameters, such as the acetate/propionate ratio and total volatile fatty acid production (Al-Jumaili *et al*., 2017).

Similarly, the effect of *Lotus pedunculatus* on the ruminal methanogens *Methanobrevibacter ruminantium* strains YLM-1 and DSM1093 showed that condensed tannins can reduce methanogenesis both indirectly —by lowering hydrogen production and forage digestibility— and directly, through inhibitory effects on methanogens (Tavendale *et al*., 2005). Cushnie and Lamb (2005) reported the antimicrobial activity of flavonoids, such as quercetin, partly through inhibition of DNA gyrase. Other proposed mechanisms include disruption of cytoplasmic membrane function by sophoraflavanone G and epigallocatechin gallate, and inhibition of energy metabolism by licochalcones A and C. Flavonoids can therefore interact positively or negatively with ruminal microorganisms.

Broudiscou *et al*. (2002) examined extracts of 13 plants selected for high flavonoid content: *Lavandula officinalis* and *Solidago virgaurea* stimulated fermentation, while *Equisetum arvense* and *Salvia officinalis* decreased methanogenesis. Consequently, some flavonoid-containing plants may reduce methane production while stimulating microbial metabolism; however, the effects of flavonoids on ruminal protozoa remain unclear (Patra and Saxena, 2010).

The findings of this study suggest that biofertilization is a promising strategy to increase secondary metabolite concentrations in forage, thereby indirectly reducing methane production in the rumen. This approach may complement other strategies aimed at mitigating greenhouse gas emissions from livestock production.

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BIOFERTILIZACIÓN DE SORGO FORRAJERO PARA INCREMENTAR LOS NIVELES DE POLIFENOLES CON POSIBLE EFECTO ANTIMETANOGÉNICO

Arely Guerrero-De Lira, Cristian Lizarazo-Ortega, María Cristina Hernández-Jiménez, Régulo Ruíz-Salazar, José Hernández-Mendoza, Ana González-Pérez y Guadalupe Rodríguez-Castillejos

RESUMEN

El metano es uno de los principales gases que contribuyen al calentamiento global ya que atrapa más calor que el dióxido de carbono en la atmósfera; el ganado produce un porcentaje significativo de este gas, por lo que una alternativa viable es reducir la metanogénesis ruminal mediante una alimentación rica en polifenoles. En el presente estudio se evaluó el incremento de compuestos polifenólicos en sorgo forrajero, dado que este cultivo se utiliza comúnmente como alimento para el ganado. Se aplicaron cuatro tratamientos: una mezcla de Bacillus *sp.,* Azospirillum brasilense*,* Trichoderma*, una combinación de hongos y bacterias y un grupo control. Estos tratamientos se probaron en siete variedades de sorgo forrajero cultivadas en México (Cañaveral, Sureño, MXSE45, Tehua, Cañero, MXSE2 y MXSE19). A los 20 y 56 días después de la siembra se midieron parámetros morfoagronómicos (altura de planta, diámetro de tallo y actividad fotosintética), así como las concentraciones de fenoles, flavonoides y taninos. La variedad MXSE19 presentó los valores más altos de altura, peso de planta y concentración de fenoles, siendo la combinación con* Trichoderma *la más efectiva. Las variedades Tehua y Cañero mostraron los valores más bajos en todos los tratamientos. El uso de biofertilizantes en forrajes representa una estrategia potencial para incrementar la concentración de polifenoles, compuestos con actividad antimetanogénica.*

BIOFERTILIZAÇÃO DE SORGO FORRAGEIRO PARA AUMENTAR OS NÍVEIS DE POLIFENÓIS COM POSSÍVEL EFEITO ANTIMETANOGÊNICO

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RESUMO

O metano é um dos principais gases que contribuem para o aquecimento global, pois retém mais calor do que o dióxido de carbono na atmosfera; o gado produz uma porcentagem significativa desse gás, e uma alternativa viável é reduzir a metanogênese ruminal por meio de uma alimentação rica em polifenóis. No presente estudo avaliou-se o aumento de compostos polifenólicos em sorgo forrageiro, dado que essa cultura é comumente utilizada como alimento para o gado. Foram aplicados quatro tratamentos: uma mistura de Bacillus *sp.,* Azospirillum brasilense*,* Trichoderma*, uma combinação de fungos e bactérias e um grupo controle. Esses tratamentos foram testados em sete variedades de sorgo forrageiro cultivadas no México (Cañaveral, Sureño, MXSE45, Tehua, Cañero, MXSE2 e MXSE19). Aos 20 e 56 dias após o plantio, foram medidos parâmetros morfoagronômicos (altura da planta, diâmetro do colmo e atividade fotossintética), bem como as concentrações de fenóis, flavonoides e taninos. A variedade MXSE19 apresentou os maiores valores de altura, peso da planta e concentração de fenóis, sendo a combinação com* Trichoderma *a mais eficaz. As variedades Tehua e Cañero mostraram os menores valores em todos os tratamentos. O uso de biofertilizantes em forragens representa uma estratégia potencial para aumentar a concentração de polifenóis, compostos com atividade antimetanogênica.*