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Integration of the Controlled Production of Huitlacoche (*Ustilago maydis* + *Zea mays*) in the Traditional Milpa System from Guerrero, Mexico

Sirilo Suastegui-Cruz [1,2], Daniel Martínez-Carrera [2,*], Miguel Sánchez [2], Myrna Bonilla [2,3], Ivan Castillo [2,3], Alfredo Morales [2], Nora Fernández [2], Beatriz Petlacalco [2] and Wilfrido Martínez [2]

[1] Program on Postdoctoral Stays for Mexico, Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT), Mexico City 03940, Mexico; sirilo_sc@uagro.mx
[2] Centre of Biotechnology of Medicinal, Functional and Edible Mushrooms (CB-HCFM), Colegio de Postgraduados (CP), Campus Puebla, Boulevard Forjadores de Puebla No. 205, Puebla 72760, Mexico; msanchez@colpos.mx (M.S.); myrna@colpos.mx (M.B.); castillo.ivan@colpos.mx (I.C.); morales.alfredo@colpos.mx (A.M.); fernandez.nora@colpos.mx (N.F.); petlacalco.beatriz@colpos.mx (B.P.); martinez.wilfrido@colpos.mx (W.M.)
[3] Postgraduate Program on Strategies for Regional Agricultural Development (PROEDAR), Campus Puebla, Colegio de Postgraduados (CP), Boulevard Forjadores de Puebla No. 205, Puebla 72760, Mexico
* Correspondence: dcarrera@colpos.mx

Abstract: Food security has been an essential priority for decades due to its direct impact on human development. Despite significant advances in meeting basic food needs, food security remains a global challenge. In Mexico, the sustainable traditional polyculture system known as milpa allows synergy among three or more food sources, increasing the economic income for peasants. We integrated the controlled production of huitlacoche (*Ustilago maydis* + *Zea mays*) galls, traditional food consumed since pre-Hispanic times, in a milpa from Las Ánimas community, Tecoanapa Municipality, Guerrero, Mexico, in order to improve food diversity, productivity, income, and food security. Two contiguous plots were evaluated: the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche). Different variables were assessed including weight and size of maize ears and huitlacoche galls; degree of huitlacoche infection; production and income of maize (*Zea mays*), beans (*Phaseolus* spp.), squash (*Cucurbita* spp.), and huitlacoche; and sustainability indicators, maize grain sales, type of maize seed, huitlacoche sales, beans sales, and squash sales during the dry season. The integration of the controlled production of huitlacoche improved productivity and net incomes from the traditional milpa. The traditional milpa system (TMS) had an income of USD $111.00 from maize grains, while the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) had an income of USD $409.00 from galls. This is reflected in the cost/benefit ratio, in which milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) was 1.48, while the traditional milpa system (TMS) was not profitable (0.91). Sustainability indicators show that the controlled production of huitlacoche galls contribute to food diversity, productivity, and income in the traditional milpa system, based on the association of maize, beans, squash, and huitlacoche, showing potential for improving food security in rural communities.

Keywords: huitlacoche; sustainability indicators; traditional milpa system; yields

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1. Introduction

Food security has been a key priority for the FAO (Food and Agriculture Organization) for decades as it contributes to human development [1]. Despite significant progress in meeting basic food needs, food insecurity persists globally and affects hundreds of millions of people [2]. Worldwide, food production can only meet the needs of less than one-third of the population due to unequal distribution that limits access and drives up product prices [3]. Producing food that nourishes the population is crucial for health, child development, economic productivity, poverty reduction, social stability, and environmental sustainability [4].

Population growth and economic development have intensified the demand for food, placing additional pressure on production processes [5], leading to the practice of monoculture, which contributes to soil degradation by depleting essential macro-nutrients needed by plants, causing infertility and compromising the long-term sustainability of the agricultural system [6]. In this context, the traditional milpa system (TMS) faces increasing vulnerability. It is a vital system developed, adapted and used in Mexico, and several other countries from Central America [7–11]. The traditional milpa system (TMS) is a polyculture primarily consisting of maize (*Zea mays* L.), beans (*Phaseolus* spp.), and squash (*Cucurbita* spp.), but it can also include endemic species from each region contributing to the diet of Mexican peasants [10].

The milpa system has the capacity to maintain and regenerate soil fertility by combining maize, beans, and squash, leveraging the synergy of species involved. Maize acts as a support for bean vines, while beans fix nitrogen in the soil. Squash, with its leaves, covers the soil, reducing erosion and maintaining moisture, thus improving soil biodiversity and nutrient retention, reducing the use of chemical fertilizers, and making efficient use of available resources [12–14]. The traditional milpa system (TMS) is a food source as it contributes different nutrients and enhances the nutritional quality of peasants' diet through its variety of products [6]; it also generates additional useful by-products, such as fodder [15]. This crop diversity ensures the availability of at least one product, preventing total losses even during environmental crises [16].

It is evident that natural phenomena, such as high temperatures and drought, affect the production of the traditional milpa system (TMS) [17,18]. Drought, in particular, reduces the availability of water for agriculture during the dry season, making it essential to have a high technology irrigation system to optimize water use in agriculture [19–22]. The traditional milpa system (TMS) stands out as the only system capable of adapting to various climatic, economic, and cultural pressures, an aspect that has been studied in terms of vulnerability [23–25]. Although the milpa system is traditionally sustainable and culturally valuable, the consequences of climate change and current low economic yields do not always guarantee a profitable income for peasants [26]. For this reason, it is crucial to explore biotechnological innovations that enhance productivity and adapt to current challenges, thereby ensuring the economic viability and resilience of the system [27,28]. The milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) is a viable alternative for improving productivity and income from the traditional milpa system (TMS).

Ustilago maydis, known as huitlacoche in Mexico, is a plant pathogenic fungus that attacks the maize plant (*Zea mays*); however, its natural occurrence in the crop is rare. The development of a biotechnological process developed by the authors ensures the controlled production of huitlacoche (*Ustilago maydis* + *Zea mays*) galls, allowing its intensive cultivation through the inoculation of sporidia directly into the young ear of maize [27]. In Mexico, there are references to the consumption of wild huitlacoche as a food since pre-Hispanic times. Considered a delicacy in the Mexican cuisine and some restaurants in the USA, it has a good functional and nutritional value containing protein, essential fatty acids, carbohydrates, and phenolic compounds [27]. Huitlacoche is highly valued in the Mexican cuisine through a variety of traditional dishes, and its commercialization in local, national, and international markets offers economic opportunities for farmers [29–34].

The controlled production of huitlacoche galls (TMS + Huitlacoche) within the traditional milpa system (TMS) aims to integrate traditional knowledge and biotechnological processes to enhance productive and economic performance of peasants. This approach will provide incentives for young peasants to adopt innovative agricultural practices, benefiting sustainable food production, as well as increasing basic scientific research [35]. In this research, we assessed and compared maize yields, quantity and quality of galls, as well as main sustainability indicators during the dry season of the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) at Las Ánimas, Tecoanapa Municipality, Guerrero, Mexico. Food diversity, productivity, income, and food security significantly improved in the traditional milpa system, showing good potential for strengthening sustainability.

2. Materials and Methods

The research was performed during the period January–May 2023, in the rural community of Las Ánimas, Tecoanapa Municipality, Guerrero, Mexico. The community is located at coordinates 99°19′07″ W longitude and 16°58′22″ N latitude, at an altitude of 660 m above sea level. The climate is warm subhumid, with an average temperature of 31 °C and annual precipitation of 1200 mm, abundant rainfall in the summer. The orography consists of slopes and inclines [36]. The area of study is referred to as the Site, where (A) represented the traditional milpa system (TMS), while (B) represented the milpa integrating the controlled production of huitlacoche galls (TMS + Huitlacoche), as can be seen in the Figure 1 using the ArcGIS 10.3 software (Esri, Redlands, CA, USA).

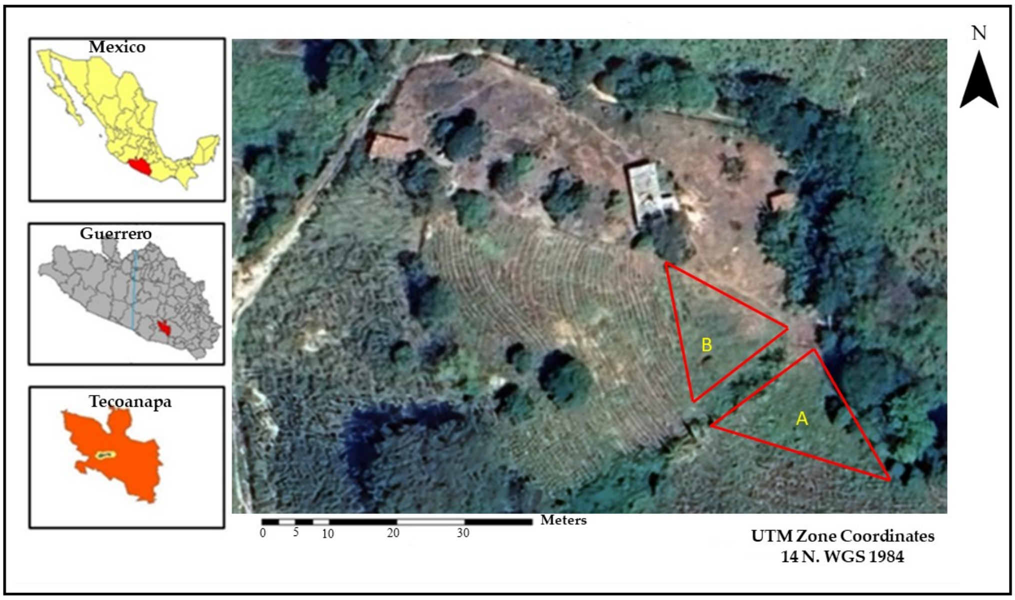


Figure 1. Geographic location of experimental plots in Las Ánimas, Tecoanapa Municipality, Guerrero, Mexico.

2.1. Experimental Design

The traditional milpa system (TMS) and the milpa integrating the controlled production of huitlacoche galls (TMS + Huitlacoche) had an area of 0.035 ha. In each block, the following were planted: maize (1200 plants) Asgrow® México A-7573 (Bayer, Mexico city, Mexico) [30], beans (324 plants), and squash (120 plants) of local varieties from native seeds selected by peasants each year [31]. The distance was 80 cm between rows and 40 cm between each plant, three maize seeds being placed per sowing in every hole made with a stick and covered up with soil. The beans were planted in the middle of maize rows at a distance of 40 cm between each hole, and 80 cm between bean rows, where four seeds were placed per every hole. The squash was planted around the maize and bean crops, at a distance of 2 m between each plant. The terrain has a 10% slope, being considered moderate hillside cultivation [31] (Figure 2).

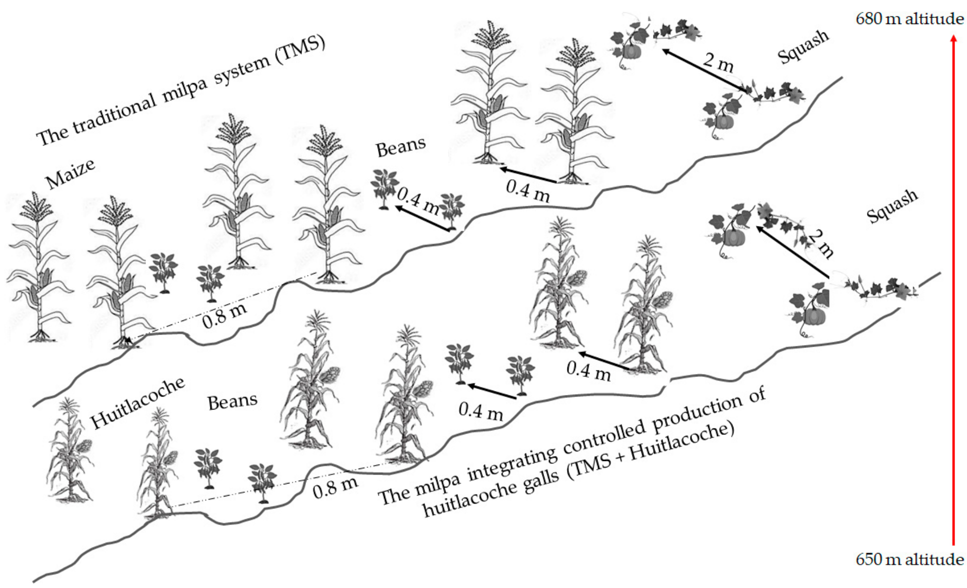


Figure 2. Schematic outline of two contiguous experimental plots studied (altitude: 650–680 m): the traditional milpa system (TMS) and the milpa integrating the controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Municipality of Tecoanapa, Guerrero, Mexico.

Furrow irrigation was used in experimental plots, the water obtained from a nearby well. Irrigation was performed during phenological stages when maize required moisture to continue its biological cycle [33].

Fertilization was carried out manually with organic compost consisting of pig and chicken manure. The first application was made after 20 days, the second after 45 days, and the third after 60 days of planting. For the control of whitefly (Aleyrodidae) pests, the organic insecticide MicroBST Yoliza® (University of Puebla, Puebla, Mexico) was applied 30 days after planting.

The plant began forming young ears of maize approximately 120 days after planting. During this period, *Ustilago maydis* (DC.) Corda was inoculated using an automatic self-filling syringe of 2 mL (HSW Eco-matic®, Tuttlingen, BW, Germany), administering two 0.5 ml injections into every young ear of maize. The inoculum contained 1×10^6 viable sporidia, corresponding to compatible strains CP-436 and CP-437, deposited at the P-ICTI-1 Platform for Genetic Resources from the Centre of Biotechnology for Medicinal, Functional and Edible Mushrooms (CB-HCFM).

To monitor Environmental conditions during the maize phenological cycle were monitored by a Davis Vantage Pro2® weather station (Davis Instruments, Hayward, CA, USA), recording maximum and minimum temperatures, precipitation, and relative humidity.

2.2. Data Collection

Harvesting was carried out manually, selecting 81 inoculated ears of maize with well-developed galls (huitlacoche), as well as 81 mature ears of maize not inoculated. The quantitative characterization of huitlacoche was made according to Castañeda de León et al. [37], while maize characterization according to Paranhos et al. [33]. The morphological characteristics of infected and non-infected maize ears from experimental plots were studied, including those from the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), as follows: maize ears with bracts (g); maize ears without bracts (g); fresh weight of galls or grains (g); dry weight of galls or grains (g); high-quality galls or grains (g); low-quality galls or grains (g); length and width of maize ears (cm); and length and width of huitlacoche galls or maize grains (cm). These quantitative variables were converted to qualitative variables by classifying them as follows: the morphological characteristics of maize ears from experimental plots, the traditional milpa system (TMS), and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) performed using quantitative data from huitlacoche infection (ear size, young ear of maize, appearance of young ears, degree of infection, galls produced (total infection), galls covered by bracts, external galls on maize bracts, and internal galls in maize bracts). The maize ears were classified according to the following variables: maize ear with bracts (g); maize ear without bracts (g); length of maize ear (cm); and width of maize ear (cm); galls and grains from experimental plots studied: fresh weight of galls or grains (g); weight of 10 galls or grains (g); high quality of galls or grains (g); low quality of galls or grains (g); length of galls or grains (cm); and width of galls or grains (cm).

2.3. Data Processing

The representative sample was n = 81 for both maize and huitlacoche. The data were processed using the SAS® (SAS Institute, Cary, NC, USA) 9.0 software, with mean comparisons performed using Tukey's method with $\alpha = 0.05$.

For qualitative analysis, the studied variables were converted from quantitative to qualitative data, establishing ranges for categorization using SPSS (Statistical Package for the Social Sciences) 20 software (IBM, Armonk, NY, USA). Both qualitative and descriptive variables underwent frequency analysis to establish later a guide for the morphological description of maize and huitlacoche. Subsequently, the income data obtained from the traditional milpa (TMS) and from the incorporation of huitlacoche (TMS + Huitlacoche) were collected, and they were analyzed per unit area. The product price in the market was investigated, recorded, and multiplied by the sales volume. The conversion from Mexican pesos to U.S. dollars was performed according to the exchange rate from April 2023 [34]. Data were processed using the SPSS 20 software.

To evaluate sustainability, the methodology for assessing natural resource management systems (MESMIS) was used [38]. A survey was applied to the household studied, and participatory monitoring was conducted from the start of planting until the sale of the products. Incomes from milpa byproducts were also assessed (maize fodder).

Using the collected information, critical points and the most relevant indicators were defined and associated to sustainability attributes of the traditional milpa system. The evaluation of indicators was carried out through survey analysis and participatory observation. This allowed the integration of responses from household members. Quantitative values were expressed as low, medium, or high based on the methodology established by Astier et al. [39], modified by González-Flores et al. [40]. These levels were established for the household studied, and classified as follows:

(a) Production cost, maize sales, and type of seed. These indicators were evaluated quantitatively, establishing three levels of assessment (profitable, very profitable, not profitable), determined through the survey. Numeric values were assigned to each level: profitable = 0–3, very profitable = 3–6, and not profitable = 6–10.
(b) Family and labor implications. Qualitative values (low, medium, high) were assigned to these indicators. Values for sub-indicators (1–10) were established and then averaged.
(c) Pests and diseases; and fertilization. The evaluation was based on the presence or absence of these factors, as risk factors that could affect the traditional milpa system (TMS) with the integration of huitlacoche (TMS + Huitlacoche). A value of 10 was assigned to the absence of both factors, 5 for the presence of one factor, and 0 for the presence of both factors.
(d) Huitlacoche, beans, and squash. These indicators were evaluated quantitatively, establishing three levels of assessment (profitable, very profitable, not profitable), determined through the survey. Numeric values were assigned to each level: profitable = 0–3, very profitable = 3–6, and not profitable = 6–10.

3. Results

The characteristic galls induced by *Ustilago maydis* appeared 20 days after inoculation (Figure 3) in the inoculated young ears of maize at the milpa integrating controlled production of huitlacoche (TMS + Huitlacoche). The *Ustilago maydis* galls were not observed or recorded at the traditional milpa system (TMS).

Figure 3. (**A**) Characteristic galls induced by *Ustilago maydis* in the milpa integrating controlled production of huitlacoche (TMS + Huitlacoche); (**B**) The traditional milpa system (TMS) without the presence of *U. maydis*.

3.1. Climatic Conditions

During the infection period (1–15 days after inoculation), temperatures ranged from a minimum of 16 °C to a maximum of 35 °C. These temperatures were similar to those recorded during the harvest period (16–30 days after inoculation), with a minimum of 16 °C and a maximum of 32 °C. Relative humidity fluctuated between 10% and 95% during infection and from 11% to 94% during harvesting (Figure 4). These climatic conditions were optimal for the development of huitlacoche and the other crops (maize, beans, and squash).

3.2. Agronomic Characterization, Infection, and Productivity of Huitlacoche

The weight maize ears with bracts (g) having huitlacoche was 460.17 ± 117.40 g, 28.36% higher than those from non-infected maize ears with bracts, which weighed 358.49 ± 67.40 g. Maize ears without bracts (g) having huitlacoche had a weight of 350.18 ± 66.82 g, 46.77% higher than those from non-infected maize ears without bracts (238.59 ± 23.55 g). Fresh weight of galls was 254.04 ± 56.57 g, greater (90.95%) than the fresh weight of maize kernels (133.04 ± 4.79 g). The dry weight of 10 galls was 16.35 ± 8.29 g, exceeding maize kernels by 227%, which weighed 5.00 ± 0.00 g. The weight of high quality huitlacoche galls was 155.68 ± 63.54 g, which was 77.83% higher than those of low quality (87.54 ± 54.49 g). The values obtained from samples of the traditional milpa system (TMS), in comparison with those from the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), showed significant differences (Figure 5).

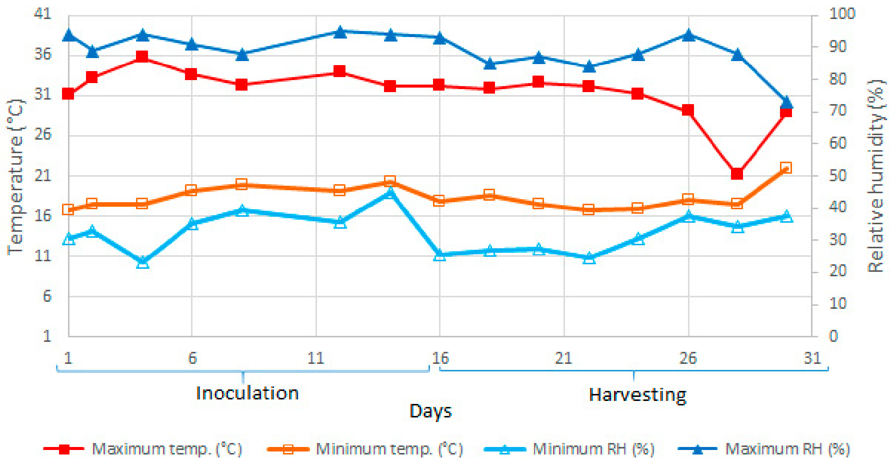
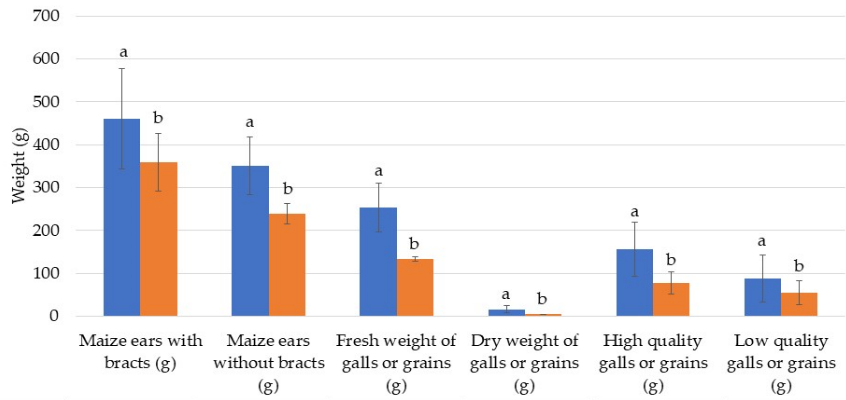


Figure 4. Temperature and relative humidity (maximum and minimum) during the production cycle of the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Municipality of Tecoanapa, Guerrero, Mexico.

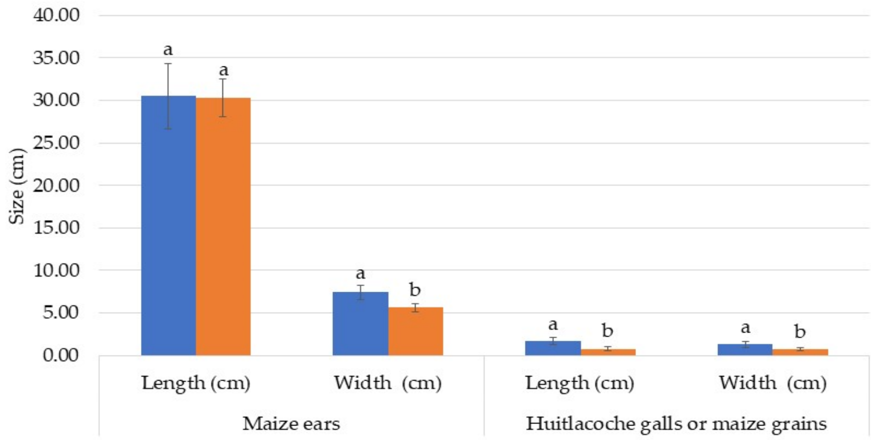


Huitlacoche	460.17 ± 117.40 a	350.18 ± 66.82 a	254.04 ± 56.57 a	16.35 ± 8.29 a	155.68 ± 63.54 a	87.54 ± 54.49 a
Maize	358.49 ± 67.40 b	238.59 ± 23.55 b	133.04 ± 4.79 b	5.00 ± 0.00 b	77.53 ± 25.75 b	55.14 ± 27.82 b

Figure 5. Average weight of maize ears, maize kernels, and quality of huitlacoche galls from experimental plots studied (n = 81) at the traditional milpa system (TMS), and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Municipality of Tecoanapa, Guerrero, Mexico. Means in a column showing differing letters indicate statistically significant difference, $\alpha = 0.05$. Values correspond to one-way Anova from the Tukey's multiple comparisons test.

In the case of galls, it was observed that the weight of high quality huitlacoche increased by 109% compared to maize kernels, a similar trend was recorded in the weight of low quality huitlacoche.

In general, it was observed that the performance of the milpa integrating the controlled production of huitlacoche galls (TMS + Huitlacoche) was better ($p > 0.05$) compared to the traditional milpa system (TMS). It was found that there was no significant difference in the length of the huitlacoche and maize ears [41,42]; however, the width of maize ears with huitlacoche was greater than those of non-infected maize ears. Additionally, the galls showed significant differences compared with maize kernels in the variables of length and width (Figure 6).



	Length (cm)	Width (cm)	Length (cm)	Width (cm)
Huitlacoche	30.51 ± 3.87 a	7.44 ± 0.82 a	1.66 ± 0.41 a	1.32 ± 0.38 a
Maize	30.28 ± 2.22 a	5.62 ± 0.51 b	0.79 ± 0.23 b	0.76 ± 0.21 b

Figure 6. Length and width of maize ears, galls, or kernels from experimental plots studied (n = 81) in the traditional milpa system (TMS), and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Municipality of Tecoanapa, Guerrero, Mexico. Means in a column showing differing letters indicate statistically significant difference, $\alpha = 0.05$. Values correspond to one-way Anova from the Tukey's multiple comparisons test.

In the results of the morphological description, we found that maize ears infected with huitlacoche were large, there was the initial presence of healthy young ears of maize, gall production was >10%, the infection grade was G3 (total infection), and there was no appearance of internal or external galls on the bracts confirming the successful inoculation of *Ustilago maydis* (Table 1).

Table 1. Morphological characteristics of maize ears from experimental plots studied, the traditional milpa system (TMS), and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

Variables	TMS +Huitlacoche (n = 80)			TMS (n = 81)		
	Feature	**Frequency (n)**	**Percentage (%)**	**Feature**	**Frequency (n)**	**Percentage (%)**
Ear size	Large	47	58.8	Medium	41	50.6
Young ear of maize	Presence	73	91.3			
Appearance of young ears	Healthy	80	100	-	-	-
Degree of infection	G3	67	83.8	-	-	-
Galls produced (total infection)	>10%	74	92.5	-	-	-
Galls covered by bracts	Semi-open	41	51.3	-	-	-
External galls on maize bracts	Absence	80	100	-	-	-
Internal galls in maize bracts	Absence	56	70	-	-	-

Degree of infection: Severity grade on maize ear, ranging from G0 = no infection, G1 = minimal infection, G2 = more than half, and G3 = total infection.

In the description of maize ears, the variable "with bracts" showed a moderate weight range (343–516 g) for TMS + Huitlacoche, while a low weight range (236–335 g) for TMS. For the variable "without bracts", a moderate weight range was recorded. Regarding length and width variables, the length of maize ears in TMS + Huitlacoche was short (18–26 cm), while that for TMS was medium (29–32 cm). In terms of width, it was medium (6–8 cm) in TMS + Huitlacoche, while large (5–6 cm) in TMS (Table 2).

Table 2. Maize ears from experimental plots studied, the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

Variables	TMS + Huitlacoche (n = 80)			TMS (n = 81)		
	Feature	Frequency (n)	Percentage (%)	Feature	Frequency (n)	Percentage (%)
Maize ear with bracts (g)	Moderate (343–516 g)	45	56.3	Low (236–335 g)	36	44.4
Maize ear without bracts (g)	Moderate (296–408 g)	48	60	Moderate (214–260 g)	63	77.8
Length of maize ear (cm)	Short (18–26 cm)	79	98.8	Medium (29–32 cm)	41	50.6
Width of maize ear (cm)	Medium (6—8 cm)	60	75	Large (5–6 cm)	78	96.3

In the description of galls, the length was medium (1–2 cm) in TMS + Huitlacoche, while that of maize grains in TMS was medium (0.7–1 cm). The width of galls was medium (1.1–1.7 cm), and the width of maize grains was high (0.9–1.2 cm). The fresh weight of the galls had a low range (171–271 g), and this was so also for the maize grains (125–131 g). The weight of 10 galls in TMS + Huitlacoche showed a low range (4–16 g). The weight of high quality galls (100–200 g) was medium in TMS + Huitlacoche, and this was so also for maize grains (46–92 g) in TMS. The weight of low quality galls showed a low range (0–86 g), and low quality maize grains (43-86 g) had a medium range (Table 3).

Table 3. Galls and grains obtained from experimental plots studied, the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

Variables	TMS + Huitlacoche (n = 80)			TMS (n = 81)		
	Feature	Frequency (n)	Percentage (%)	Feature	Frequency (n)	Percentage (%)
Fresh weight of galls or grains (g)	Low (171–271 g)	45	56.3	Low (125–131 g)	34	42
Weight of 10 galls or grains (g)	Low (4–16 g)	50	62.5	-	-	-
High quality of galls or grains (g)	Medium (100–200 g)	57	71.3	Medium (46–92 g)	65	80.2
Low quality of galls or grains (g)	Low (0–86 g)	42	52.5	Medium (43–86 g)	62	76.5
Length of galls or grains (cm)	Medium (1–2 cm)	44	55	Medium (0.7–1 cm)	38	46.9
Width of galls or grains (cm)	Medium (1.1–1.7 cm)	56	70	High (0.9–1.2 cm)	69	85.2

3.3. *Incomes Obtained from the Experimental Plots Studied, the Traditional Milpa System (TMS) and the Milpa Integrating Controlled Production of Huitlacoche Galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico*

After characterization, the harvest of the crops in the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) was conducted. For the beans, an income of USD $50.00 was obtained, and for the squash, an income of USD $36.00 was obtained. The yields were equivalent for both plots, as shown in Table 4.

Representative yields of the traditional milpa system (TMS) were as follows: the maize in grains, USD $111.00; fresh maize leaves, USD $67.00; beans, USD $50.00; and squash, USD $36.00. The initial investment for the control plot was USD $273.00, with total income from the plot amounting USD $264.00 (Figure 7).

Table 4. Average production, price, and incomes obtained from beans and squash in experimental plots studied, the traditional milpa system (TMS) and the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

Product	Seeds (No.)	Production (kg)	Price (USD/kg)	Net Income (USD)
Beans	234	140.4	$2.80	$50.00
Squash	120	56.46	$1.56	$36.00

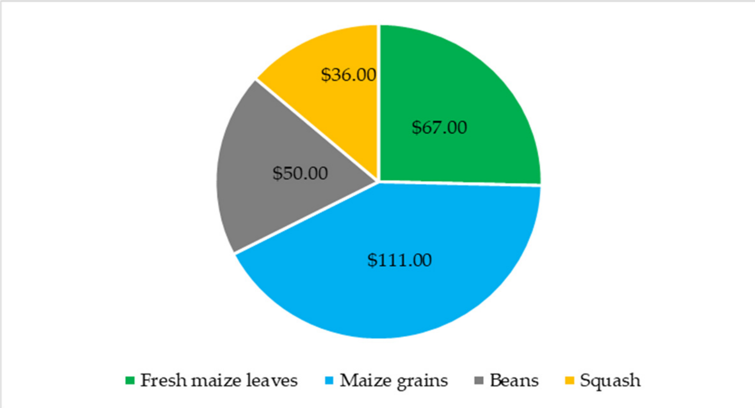


Figure 7. Incomes (USD) obtained from selling representative samples of fresh maize leaves (fodder), maize grains, beans, and squash produced in the experimental plot studied of the traditional milpa system (TMS) in Las Ánimas, Guerrero, Mexico.

In the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), 92 kg of huitlacoche was obtained, sold in the same locality at a price of USD $4.44 per kg, resulting in an income of USD $409.00. Fresh maize leaves generated a total income of USD $67.00; 140.4 kg of beans generated USD $50.00; and 56.46 kg of squash generated an income of USD $34.00. It is noteworthy that, during this period, all products were marketable, especially those that are edible due to their freshness, which increases their market value (Figure 8).

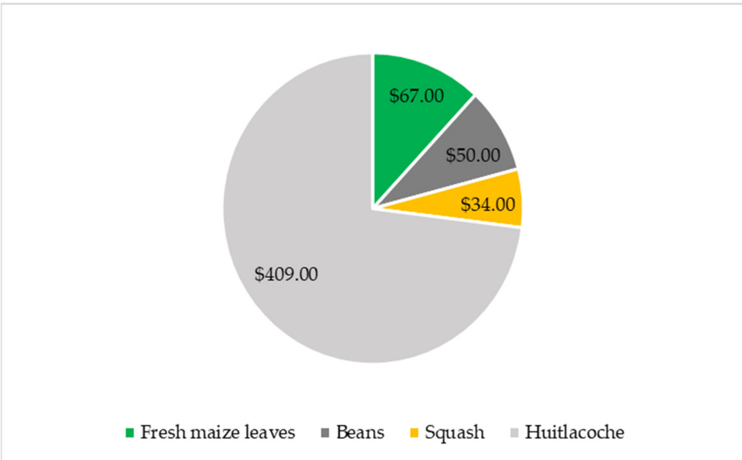


Figure 8. Incomes (USD) obtained from selling representative samples of fresh maize leaves (fodder), maize grains, beans, and squash produced in the experimental plot studied of the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

The financial analysis indicated a cost–benefit ratio of 0.91 for the traditional milpa system (TMS), showing a negative economic feasibility, excepting when the production of food for own consumption is considered in family agriculture (Table 5).

Table 5. Financial analysis of the traditional milpa system (TMS) for small peasants in family agriculture owning 0.035 ha land in Las Ánimas, Guerrero, Mexico. MXN: Mexican pesos. USD: United States dollars.

Variable	**Component**	**Amount**	
		MXN	**USD**
Cost of Production	Maize production, harvesting, and selling	$4500.00	$250.00
	Various costs	$450.00	$25.00
	Subtotal	$4950.00	$275.00
Incomes	Gross income (900 kg maize)	$4500.00	$250.00
Net income		−$450.00	−$25.00
The cost–benefit (C/B) ratio		0.91	
Consumer price		$5.00	$0.28

The financial analysis indicated a cost–benefit ratio of 1.48 for the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), showing a positive economic feasibility, as well as improving crop diversity, productivity and food security (Table 6).

Table 6. Financial analysis of the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) for small peasants in family agriculture owning 0.035 ha land in Las Ánimas, Guerrero, Mexico. MXN: Mexican pesos. USD: United States dollars.

Variable	**Component**	**Amount**	
		MXN	**USD**
Cost of Production	Maize production, shelling, and technical assistance	$5300.00	$294.44
	Huitlacoche inoculum (1200 plants)	$1500.00	$83.33
	Postharvest	$2000.00	$111.11
	Various costs	$880.00	$48.89
	Subtotal	$9680.00	$537.78
Incomes	Income (179 kg huitlacoche)	$14,320.00	$795.55
Net income		$4640.00	$257.78
The cost–benefit (C/B) ratio		1.48	
Cost of production of 1 kg packaged huitlacoche		$54.08	$3.00
Consumer price		$80.00	$4.44

3.4. Sustainability Indicators in the Experimental Plot Studied of the Milpa That Integrates the Controlled Production of Huitlacoche Galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico

From the analyzed variables of the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in a rural family unit, most sustainability indicators are on a scale from 1 to 10, in which huitlacoche sales are at an optimal level. This means that its incorporation into the system provided social and economic benefits to peasants. This can also be inferred because huitlacoche developed very well on the type of cultivated maize seed (Figure 9). Other indicators included were maize, beans, and squash sales; family involvement in production and commercialization activities; labour; productions cost; and management of pests and diseases.

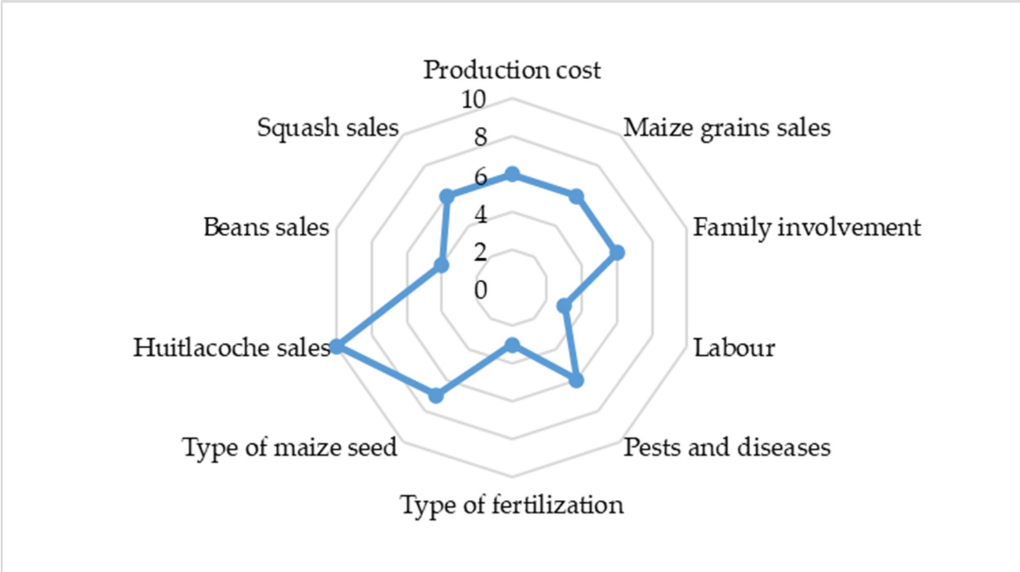


Figure 9. Sustainability indicators studied in the experimental plot of the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) in Las Ánimas, Guerrero, Mexico.

Overall, the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) had a favorable impact on the production of the plot studied during the cultivation period. This highlights the importance of diversifying the system according to the edapho-climatic conditions of the region where it is developed, especially as a viable option in response to climate change. Additionally, it is also important to promote and integrate huitlacoche into the Mexican diet. Diversifying and spreading recipes or dishes in rural areas is necessary for the integration of edible fungi into the diet of peasants, whose primary activity is agriculture.

4. Discussion

The agricultural sector in Mexico faces significant challenges, arising from the unequal distribution of income and the growing demand for food due to population growth. In this context, the production of huitlacoche (*Ustilago maydis* + *Zea mays*), an endemic food of high nutritional and economic value, offers a viable alternative to contribute to food security [43–46]. Various studies have warned that the current industrial food model tends to undervalue important natural resources, such as edible mushrooms, which underlines the importance of exploring their potential in sustainable agricultural systems [47]. Incorporating this fungus into the diet has the potential to contribute significantly to food security.

The present study, carried out in Las Ánimas, Guerrero, Mexico, shows that the warm subhumid climate with temperatures between 16 °C and 35 °C favors the production of huitlacoche [45]. The availability and efficient use of water facilitated the good development of galls on the inoculated maize ears. Efficient water management and proper crop management allowed the optimal development of huitlacoche, obtaining gross yields of 147.42 g/plant and an estimated productivity of 7.45 t/ha. These values exceeded the 6 t/ha reported by Castañeda de León et al. [37], which highlights the viability of huitlacoche production not only in this region, but also in other tropical and subtropical areas of Mexico [43], where climatic conditions are similar. These results confirm that huitlacoche can be a viable agricultural option in different geographic contexts, opening up new opportunities for local peasants [47,48].

When analyzing the comparison between the traditional milpa system (TMS) and the system that integrates the controlled production of huitlacoche galls (TMS + Huitlacoche), the data reflect a considerable improvement in terms of economic profitability and nutritional quality. TMS + Huitlacoche not only increases family income, but also provides a food with good nutritional profile. This aspect is relevant, as it shows the importance of integrating high-value crops into traditional agricultural systems. Although previous studies have demonstrated the viability of TMS with the incorporation of different crops [10], this study showed the integration of the fungal kingdom through the controlled production of huitlacoche optimizing both productive efficiency and economic benefits, as well as its potential in the Mexican agricultural context.

Ustilago maydis stands out for its interesting nutritional value, which includes high levels of protein, dietary fiber, fatty acids, minerals, and vitamins, as well as antioxidant, antimicrobial, anti-inflammatory, antimutagenic, antiplatelet, and dopaminergic properties [27]. Compared to other edible fungi, huitlacoche offers significant nutritional advantages, especially in rural regions where the population has nutritional deficiencies [45], a perspective that Astier et al. [39] refer to as a traditional agroecological approach. González-Flores et al. [40] also discussed contemporary dynamics into the system. This contribution highlights the importance of preserving traditional practices, while recognizing the benefits of adopting and adapting new technologies and innovations to ensure long-term sustainability.

The milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche) has significant contributions to agriculture and food. The highest economic profitability was obtained from huitlacoche, suggesting that its inclusion in cultivation can diversify agriculture and improve the sustainability of agricultural systems in Mexico [49]. In this way, huitlacoche production can be an effective strategy to address challenges related to adverse climatic conditions and enhance food security.

This study proposes the production of huitlacoche in the milpa system, in order to improve food security and agricultural profitability in Mexico. Huitlacoche production in the traditional milpa system not only enhances the economic profitability of family agriculture, but also offers a sustainable and nutritious option for food supply in Mexico. The integration of huitlacoche makes the system more productive and sustainable, while also improving global food security by promoting food diversification and supporting the expansion and preservation of this valuable yet underutilized fungal genetic resource [50,51]. The results of the present study revealed that huitlacoche can be fundamental to the sustainability and diversification of agricultural systems in the context of climate change and food system transformation towards healthy diets [26].

Huitlacoche production in milpa systems can be an effective strategy to improve agricultural sustainability under adverse climatic conditions, based on the association of maize, beans, squash, and huitlacoche. The inclusion of huitlacoche not only increases the profitability of the system, but also contributes to strengthening the resilience of the agroecosystem to climatic variations, which is particularly important in the context of climate change. Furthermore, diversifying the agricultural system through huitlacoche production promotes sustainability and food security, while taking advantage of an underutilized fungal genetic resource of significant social, economic and nutritional impact.

5. Conclusions

High temperatures during the dry period did not interfere with the development of huitlacoche and other food products, leading to better utilization of the plot's by-products.

The huitlacoche reached expected yields and quality standards, generating a higher income of USD $409.00 in the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), compared to USD $111.00 in the traditional milpa system (TMS). This is reflected in the cost/benefit ratio of the milpa integrating controlled production of huitlacoche galls (TMS + Huitlacoche), which is 1.48, in comparison with 0.91 of the traditional milpa system (TMS). The family unit members are aware that production from the traditional milpa system results in economic losses due to environmental problems associated to the rainy season; thus, planting the system during the dry season is a viable alternative.

Sustainability indicators show a high level of sustainability in the milpa agroecosystem integrating huitlacoche. This contributes directly to the economic feasibility, with huitlacoche standing out in the system. At the sustainability level, it strengthened economic benefits and efficiency. However, the system remains vulnerable and fragile in the social and environmental dimensions to be improved with further equitable social participation and social organization.

The controlled production of huitlacoche in the traditional milpa represents an opportunity to transform family agriculture in Mexico, raising the possibility of further research into the interactions between huitlacoche and other traditional crops within diverse local agroecosystems. It also opens the debate on how agricultural systems can better adapt to climate change conditions, ensuring both sustainability and food security.

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