

Research Article

Green Synthesis of Ag NPs Using *Ustilago maydis* as Reducing and Stabilizing Agent

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Received 12 April 2022; Revised 25 June 2022; Accepted 25 June 2022; Published 19 July 2022

Academic Editor: Brajesh Kumar

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Ustilago maydis (UM) is a fungus that grows naturally on *Zea mays*; it reduces the corn yields, and thus, it represents huge economic loss; however, it can be used as an exotic food, and in the present work, it is successfully used as a reducing and stabilizing agent for the preparation of silver nanoparticles (Ag NPs) due to its content of amino acids and biosurfactants. The effects of the concentration of UM aqueous extract, pH, and sunlight on the particle size, surface plasmon resonance, stability, and morphology of Ag NPs obtained by green synthesis were evaluated. A green reduction was observed only in presence of UM, and colloidal Ag NPs were obtained with or without the presence of sunlight; nevertheless, continuous sunlight exposure greatly increased the reaction rate. Ag NPs tend to increase in size from 153 nm to 1400 nm at a higher pH and a greater amount of UM, and also, UM tends to stabilize the Ag NPs preventing their agglomeration according to measurement of zeta potential (-10.75 ± 0.84 mV) and SEM observation; furthermore, surface plasmon resonances were more intense between 400 and 480 nm of wavelength adding greater amount of UM. This study concludes that UM not only reduces AgNO_3 but also acts as stabilizer of Ag NPs.

1. Introduction

Silver nanoparticles (Ag NPs) have been proposed for important applications such as radiosensitizers [1], solar fuel cell efficiency improvement [2], antibacterial agents [3–5], medicine [6], anticancer [5], dental biocompatibility materials, [7] and as catalysts for chemical reactions [8]. Ag NPs not only have antifungal [9], antiviral [10], and antibiotic properties against resistant bacterial strains [11] but also show debatable toxicity to human and animal cells [12–14]. Their properties depend on their size, surface structure and shape. Ag NPs can be obtained via photochemical [15] and chemical reduction [16–18]. The use of natural sources as reducing agents has been considered a part of green synthesis and has drawn attention because it is considered safe, nontoxic, cost-effective, and environment friendly. A high diversity of plant extracts, such as *Eugenia jambolana*, *Saraca asoca*, *Rhynchoctechum ellipticum*, *Malus domestica*,

Vitis vinifera, *Adansonia digitata*, *Solanum nigrum*, and *Nitraria schoberi*, soluble starch, natural rubber, tarmac, cinnamon, stem-derived callus of green apple, red apple, egg white, lemongrass, coffee, and black tea [5] have been explored as natural reducing agents because of their low cost, their effect on increasing the reaction rate, and their high efficiency to form crystalline NPs of various shapes and sizes. Proteins, enzymes, phenolic acids, and other chemicals extracted from plants not only reduce silver salts but also provide excellent antiagglomeration properties that can be further studied and used to study plants and their parts itself, according to how they form Ag NPs or other metal nanoparticles [19]. Microorganisms, such as bacteria, fungi, or edible mushroom [20], have also been used to reduce Ag^+ salts and to obtain metallic Ag NPs; to achieve this, it is necessary to cultivate them in growth medium and later use them to inoculate a liquid medium [21]. Recently, yeast extract was used to synthesize monodispersed Ag NPs with

no precipitation within a year because of the amino acids, vitamins, and carbohydrates that supply reducing and capping agents [22]. Sunlight and artificial light are photoinductors in the reduction of AgNO_3 as a precursor for Ag NPs, and it is difficult to establish the exact mechanism of how the natural extracts induce the reduction and structural conformation of Ag NPs [23–25].

On the other hand, *Ustilago maydis* (UM) is a smut fungus that belongs to Basidiomycetes; it infects maize (*Zea mays*) and represents crop loss, except in Mexico, where it is called “huitlacoche,” and it is an exotic food with a unique appearance and flavor and a valuable nutritional source [26]. The UM contains amino acids, fatty acids [27], vitamins, phenolic compounds [28], and biosurfactants [29] different from those contained in maize and together have the potential to reduce and stabilize the Ag NPs, as this study suggests.

The aim of this work was to obtain and characterize the Ag NPs obtained using *U. maydis* as a reducing bioagent and to evaluate the effect of its concentration, pH, and sunlight on the Ag NPs formation since there is lack of information in using *U. maydis* as a stabilizing agent nor the quantification of sunlight effect in the Ag NPs synthesis using a green reductor.

2. Materials and Methods

2.1. Materials and Reactants. UM was purchased at a local market in Santa María del Monte, Zinacantepec, Mexico. AR-grade AgNO_3 was purchased from Sigma Aldrich. The pH was adjusted to 0.1 N with NaOH and potentiometer Science Med with a glass electrode. Deionized water was used for the preparation of all solutions for this work ($0.1 \mu\text{S}/\text{cm}$ of electrical conductivity).

2.2. Synthesis of Ag NPs. UM was manually separated from corn kernels and dried at 60°C in a laboratory oven for 24 h until a constant weight was reached; then, it was manually grounded and sieved before use. Extracts were prepared with 0.1, 0.5, or 0.9 g of UM powder mixed with 50 mL of deionized water and stirred at room temperature for 10 min; then, the mixture was filtrated and the pH was adjusted to 9, 10, or 11. The reduction was performed similar to [5]; it was done by mixing 10 mL of 0.01 N AgNO_3 with 10 mL of the UM aqueous extract with or without a natural or artificial source of light. By using a lux meter, it was determined that in the absence of light (inside a dark chamber), the reaction mixture was exposed to 0–20 lux, in contrast to >10000 lux, when exposed to direct midday sunlight. As a control of the reduction reaction, 10 mL of deionized water was added to the AgNO_3 solution instead of the aqueous extract of *U. maydis*.

2.3. Sample Characterization. To monitor the rate of the reduction step, UV-Visible (UV-Vis) spectroscopy was performed (DR6000 spectrophotometer, HACH, USA) in a 0.2 cm path length quartz cuvette and scanning over a 240–900 nm wavelength range. The quartz cuvette was

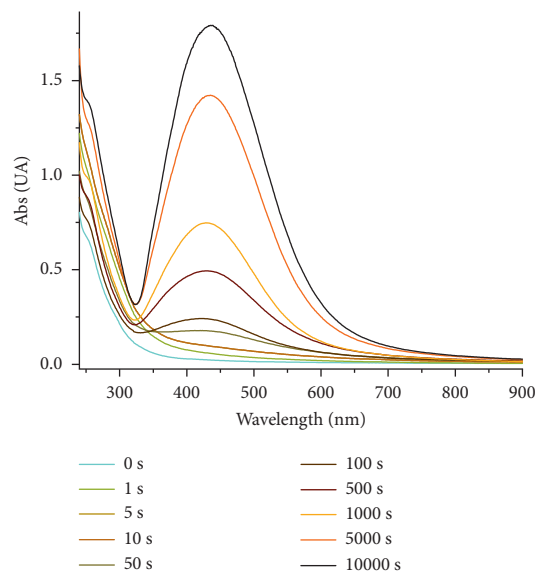


FIGURE 1: UV-Vis spectrum of Ag NPs synthesized with 0.5 g of *U. maydis* at pH 10 after a long period of sunlight exposition at room temperature.

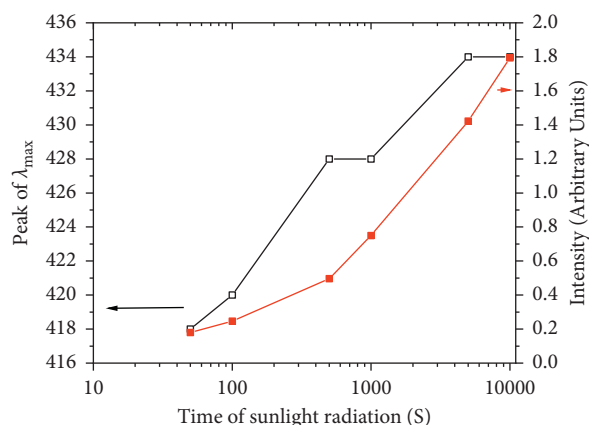


FIGURE 2: Effect of sunlight radiation on the maximum wavelength (λ_{max}) and absorbance of Ag NPs determined by UV-Vis spectrum. Reaction conditions were adjusted to 0.5 g of *U. maydis*, at pH 10 and room temperature.

cleaned constantly with acidified water (0.2% HNO_3) and rinsed thoroughly with deionized water to avoid cross-interference between readings of the samples [5].

Dynamic light scattering (DLS) was done by duplicate on a laser particle size analyzer (Litesizer 500, Anton-Paar, Switzerland) with a polyethylene cuvette with 1 cm of path length, a light source of 40 mW and 658 nm in automatic measurement angle, and the 0.159 as refractive index and 4.3 m^{-1} as the absorption coefficient, assuming that the sample material was reduced silver and water was the solvent with a refractive index of 1.33 [5].

Zeta potential at 25°C was performed on the same particle analyzer by electrophoretic light scattering with an Omega cuvette (polycarbonate cuvette with two gold-coated electrodes located at the ends of a U-shaped capillary tube) using the Smoluchowski approximation [5].

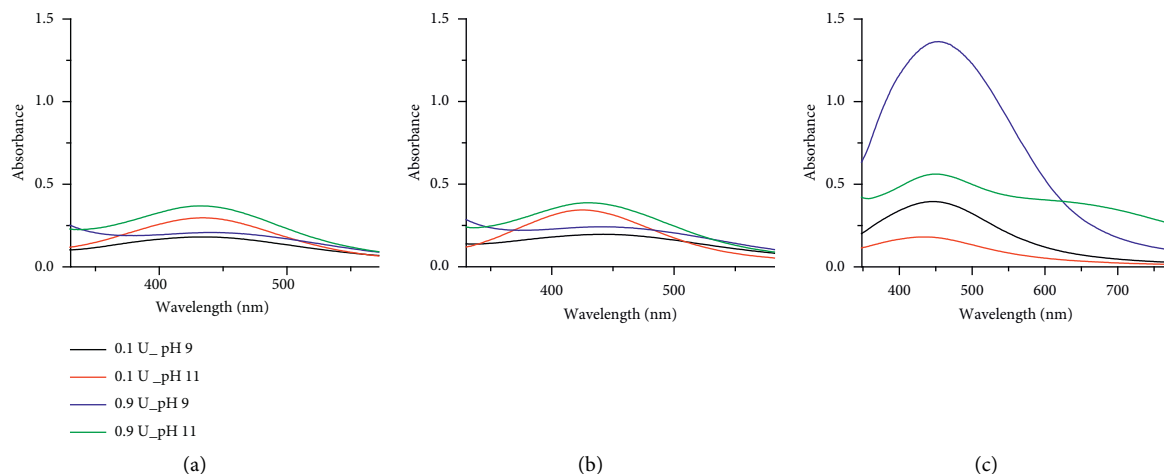


FIGURE 3: UV-Vis spectrum of Ag NPs obtained under 0.1 g or 0.9 g of *U. maydis* at pH 9 and pH 11 after (a) 100 seconds of sunlight exposure at room temperature, (b) 100 seconds of sunlight exposure and 10 min of reaction in the dark, and (c) 100 seconds of sunlight exposure and 6 days of reaction in the dark.

Scanning electron microscopy (SEM) was acquired from dried drops of the samples left on an aluminum disk sample holder that was mounted into the electronic microscope (JSM-6510LV, JEOL, Japan) at a high vacuum and was observed at an acceleration voltage of 5–8 kV with a secondary electron detector (SED). Energy dispersive spectrometry (EDS) analysis was performed with QUANTAX-EDS X-FLASH 6L 30 (BRUKER) detector, and elements were quantified with ESPRIT software [5].

3. Results and Discussion

3.1. Effect of Sunlight. The sunlight effect on AgNO_3 under conditions of 0.5 g de UM at 10 pH was evaluated, and it is shown in Figures 1 and 2. The first peak of the maximum wavelength was achieved after 50 seconds of sunlight exposure, and the absorbance of the reduced AgNO_3 started to increase; a redshift on surface plasmon resonance was observed relatively proportional to the time of sunlight exposure. The well-defined absorption in the region between 400 and 480 nm is related to Ag NPs formation, the increase in absorbance is related to the number of Ag NPs formed, while the increase in maximum wavelength or redshift is related to an increase in the particle size of the Ag NPs [30–32]. The fact that both maximum wavelength and absorption increase is because NPs agglomeration occurs. First, the number of Ag NPs escalates and accumulates in the reaction volume, and the NPs continue to assemble with each other into clusters, as shown in Figure 2 which indicates the displacement increment of the maximum wavelength absorption of the nanoparticles during the synthesis (black curve) and the increase of absorbance intensity (red curve).

Furthermore, the experience in the dark and after 24 hours of reaction was followed. A spectrum revealing a maximum wavelength at 384 nm and 0.159 of absorbance intensity was obtained, which can be related with smaller and less abundant Ag NPs. Also, it was assessed if sunlight is required to start the reaction or if it is also necessary for the

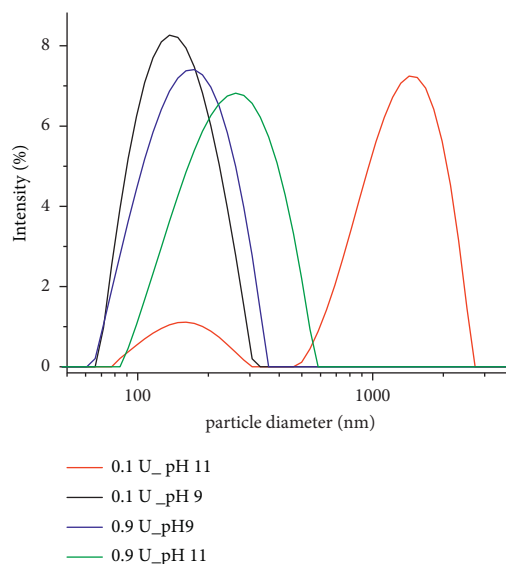


FIGURE 4: Particle diameter of Ag NPs obtained under 0.1 g or 0.9 g of *U. maydis* at pH 9 and pH 11 after 100 seconds of sunlight exposure at room temperature.

reaction to pass chemical equilibrium to reach completion. It was observed that even if the reaction occurs in the dark, changes in number and size are fewer when the reaction is kept in the dark, even with initial sunlight exposure. The vast difference between sunlight and darkness effects gave us the insight that to clearly study the effect of UM, the next reactions had to be carried out either in the absence of light for a long period of time or in sunlight for a short period of time. Hence, it was selected for 100 seconds of initial light exposure and a follow-up of up to 10 min and to 6 days to study the pH effect and the addition of UM extract to the reaction.

3.2. Effect of *U. maydis* and pH. A control reaction without UM was also studied and no reaction was observed. Aqueous extracts of 0.1 and 0.9 g of UM (0.1 U and 0.9 U, respectively)

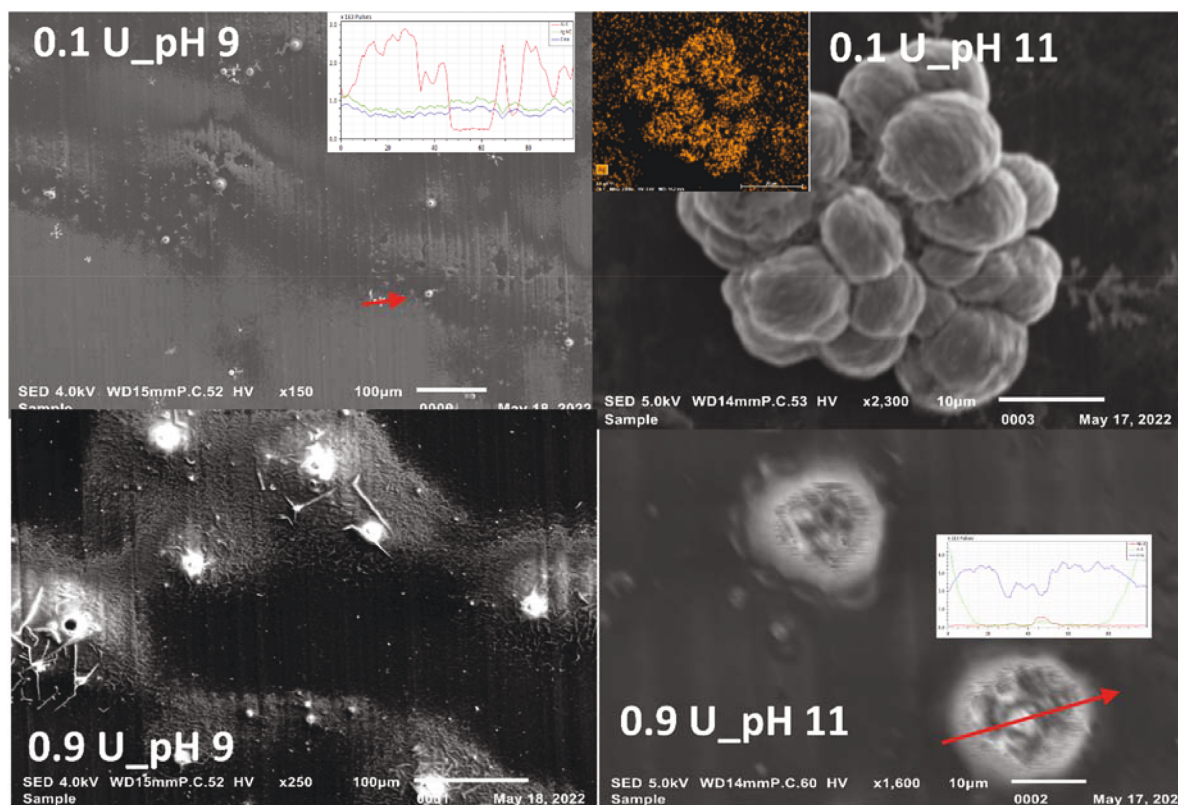


FIGURE 5: SEM of Ag NPs obtained under 0.1 g or 0.9 g of *U. maydis* at pH 9 and pH 11 after 100 seconds of sunlight exposure at room temperature with inserts that demonstrate the formation of Ag NPs, the agglomerations, and the encapsulation of Ag NPs with structures that contain C element related with UM presence.

were used at pH 9 and 11 with 100 seconds of light exposure (Figure 3(a)) followed by 10 min (Figure 3(b)) and 6 days (Figure 3(c)) in the absence of light to study the Ag NPs stability. In general, both amounts of UM and pH increases the number and size of Ag NPs. A 0.9 U extract at pH 11 had the highest absorbance intensity during the initial 100 seconds and the next 600 seconds in the dark. However, after 6 days of being maintained in the dark, the 0.9 U at pH 9 had the highest absorbance intensity around 420–450 nm, and thus, the higher production of dispersed Ag NPs. On the other hand, the UV-Vis spectrum of the reaction of 0.9 U at pH 11, which was the darkest of the four samples, had a wider and less defined band in the region of 600 nm that indicates that Ag NPs were agglomerated.

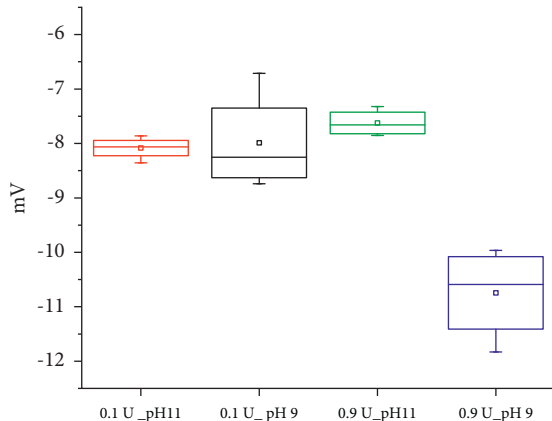
3.3. Particle Size and Stability. In Figure 4, the particle size distributions of Ag NPs are shown to analyze the effect that pH and UM aqueous extracts had on the colloidal solutions prepared after 100 seconds of sunlight exposure. The NPs obtained at pH 11 tend to increase in size, as was observed by UV-Vis spectroscopy. With extracts of 0.1 U, the difference of particle size greatly differs from pH 9 to 11 than when 0.9 U extracts are used at both pHs. Interestingly, in the particles obtained with the 0.1 U extract at pH 11, two distributions were found, a larger one with 1232 ± 389 nm average diameter and another of 121 ± 30 nm average diameter. No aggregation was observed at pH 9 with either

0.1 U or 0.9 U extracts, in whose peaks, the average diameter obtained for the nanoparticle populations was 153 ± 51 nm and 170 ± 67 nm, respectively. For 0.9 U at pH 11, the mean particle size increased to 256 ± 102 nm. The results are of interest because the increase of UM in the reaction had little impact on the increase of particle size, and it diminished the agglomeration of the Ag NPs obtained.

DLS measurements are supported by SEM microscopy observations, as shown in Figure 5; however, comparison between techniques must be done carefully as the particle size of the Ag NPs determined DLS refers to a hydrodynamic diameter, while SEM observes a particle size in the dry state, and thus, the sizes obtained by these techniques have different interpretations. In previous SEM observations, the Ag NPs were observed as bright dots with quasi-spherical morphology and an average diameter of approximately 145 ± 47 nm for 0.1 U at pH 9, 225 ± 45 nm for 0.9 U at pH 9, 4183 ± 1263 nm for 0.1 U at pH 11, and 178 ± 78 nm for 0.9 U pH 11 (Table 1). We select some patterns observed on SEM to make EDS analysis to confirm that at higher pH, Ag NPs tend to increase in size and quantity and that C atoms were found in higher presence with UM increase; for 0.1 U at pH 11, Ag NPs tend to agglomerate, and for 0.9 U at pH 11, Ag NPs tend to present as encapsulated by a biological structure that we can infer is a combination of different structures presented in UM. As can be seen, particle size determined by SEM for sample 0.1 U at pH 11 was higher than expected; it can probably be related to the sample treatment prior to the

TABLE 1: Comparison of Ag NPs particle size by DLS and SEM (after drying).

| Name (U) | pH | Zeta potential (mV) | Average diameter by DLS (nm) | Average diameter by SEM (nm) |
|----------|----|---------------------|--|------------------------------|
| 0.1 | 9 | -7.98 ± 0.91 | 153 ± 51 | 145 ± 47 |
| 0.1 | 11 | -8.08 ± 0.20 | 1232 ± 389 (1 st peak) 121 ± 30 (2 nd peak) | 4183 ± 1263 |
| 0.9 | 9 | -10.75 ± 0.84 | 170 ± 67 | 225 ± 45 |
| 0.9 | 11 | -7.62 ± 0.24 | 256 ± 102 | 178 ± 78 |

FIGURE 6: Zeta potential of Ag NPs obtained under 0.1 g or 0.9 g of *U. maydis* at pH 9 and pH 11 after 100 s of sunlight exposure at room temperature.

micrography that consists of sample drying at 50°C, which can induce agglomeration of the particles. In the micrography, this is observed as a bright cloud that tends to agglomerate.

In Figure 6, the zeta potential of the four conditions is shown as a box graph with the respective standard deviation. All samples presented a negative zeta potential with a significant difference ($p < 0.05$) for 0.9 U at pH 9 that showed the lowest negative value of -10.75 ± 0.84 mV, which is an indication of the conditions for the most stable nanoparticles obtained, which points to the fact that the increase of UM extract can confer stability to the nanoparticles, and it does not only act as a reducing agent like other green reducing agents that can be extracted from other natural sources.

Further studies must be done to identify which conditions of extraction of UM can modify its reducing and stabilizing effects on the preparation of Ag NPs via green synthesis, as well as to assess which components of the UM are responsible into confer stability.

It must be noted that the procedure presented in this work is based on Ag NPs prepared with an aqueous extract that may contain colloidal suspended solids of UM that may provide a surface interface for the cation reduction, and thus, these may work as reactive sites for stable nanoparticle growth. Also, sunlight was used for some time in the reaction, and it is composed of a wide range of wavelengths and multiple direction components that may interact differently during the reducing step of the synthesis. A direct control of the light characteristics (wavelength, incident angle, intensity, and polarization) and time exposure may result in nanoscale-controlled particle growth and accurate assembly of the nanoparticles, as other studies have shown [33].

Though different maize varieties have significant differences on UM characteristics, it is considered to have good nutritional features as it contains essential amino acids (including lysine and tryptophan) [34], fair amount of unsaturated fatty acids, glycerol, minerals, monosaccharides and polysaccharides (including glucose and fructose as the most abundant, both reducing sugars), vitamins (including vitamin C, a potent antioxidant), and abundant phenolic compound, flavonoid compounds, and chlorophylls [26, 27]. Thus, regarding the reduction reaction, it may be impossible to pinpoint a single specific compound that interacts with AgNO_3 and carries out the reduction to produce Ag NPs. Research studies exploring other reducing agents propose several mechanisms at alkaline conditions that can explain it, as Ag^+ cations can interact with hydroxide ions to form silver oxide particles that may allow for catalytic reduction of Ag^+ on their surface [35, 36]. Also, it has been demonstrated that Ag^+ cations can be reduced by aldehydes [37–39] and alkoxides [40, 41] which can be formed in organic matter containing alcohols, ketones, and aldehydes in the presence of dissolved oxygen. Even at neutral pH, it has been proposed that conversion of hydroxyls groups into aldehydes and later to carboxylic acids may be a reduction mechanism for Ag^+ [42]. Moreover, dehydration of UM may have initially produced esterification of various components that were reversibly reconverted to monosaccharides, alcohols, and fatty acids in the presence of water and then again oxidized by Ag^+ .

The stabilization of nanoparticles prevents their agglomeration and sedimentation; therefore, it allows to adjust particle sizes and shapes; for this purpose, surfactants are typically added to the reaction. It has been identified that under low nitrogen concentrations, UM produces large amounts of ustiliagic acid and ustilipid acid, two biosurfactants derived from glycolipids [29]. Furthermore, lignin, a complex polymer present in plant support tissues including corn and likely also present in UM, has been used successfully as a reducing and capping agent in Ag NPs synthesis [36] and may be part of the active compounds in UM extracts. This is relevant since not only reducing agents are of interest but also amphipathic biochemical molecules with surface active properties have several advantages in green synthesis, compared to synthetic surfactants, because the former are biodegradable and have low toxicity, and they work effectively with controlled activity at specific pH and temperatures.

4. Conclusions

Aqueous extracts of *Ustilago maydis*, natural smut of *Zea mays*, induce the reduction of AgNO_3 to obtain Ag NPs within sizes between 100 and 5000 nm, depending on the

reaction conditions. The main factors that affect the size and stability of Ag NPs are the presence of sunlight, pH, and quantity of UM aqueous extract which not only acts as a reducing agent but also confers stability to the metallic nanostructures. The analysis of this green synthesis method confirms that components in UM are effective in reducing silver nitrate and in stabilizing Ag NPs. Further studies are necessary to know which components of UM act as reducer and stabilizer.

Data Availability

The data used to support this study are included within the article.

Disclosure

The authors are part of SNI-Conacyt.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors want to thank the Universidad Tecnológica de Zinacantepec for the use of the equipment and Ana Karen Zarza Nava for the support of the realization of preliminary proofs.

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