# Silver Nanoparticles as Germination and Growth Promoters in Zucchini (*Cucurbita pepo*), Maize (*Zea mays*) and Barley (*Hordeum vulgare*)

# Nanopartículas de Plata como Promotoras de la Germinación y Crecimiento de Calabacita (*Cucurbita pepo*), Maíz (*Zea mays*) y Cebada (*Hordeum vulgare*)

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**DOI:** 10.35429/JANRE.2023.13.7.1.6 Received June 25, 2023; Accepted November 30, 2023

**Abstract**

Nanoparticles (NPs) have attracted a lot of attention due to their potential applications in biology, solar cells, and optoelectronic devices including cosmetics, biomedical science, optical devices, pharmaceutic genes (Manesh *et al*., 2010). Unlike other synthesis options, when obtaining nanoparticles by Green Chemistry, a plant extract is used as a reducing agent, often said extract can come from vegetable residues called lignocellulosic. Recent investigations have evidenced the relationship between vegetable development and nanostructured materials, identifying that a certain number of them can act as promoters of germination, seedling growth and the development of complete plants, positioning themselves as an alternative to improve agricultural production (Lopez, 2022). Therefore, the objective of this study is to develop a sustainable proposal for the production of silver nanoparticles (Np's Ag) and their application as promoters of the germination of zucchini (*Cucurbita pepo*), maize (*Zea mays*) and barley (*Hordeum vulgare*) crops in which their production, consumption and economic impact are of great importance for the Estado de México State; considering this methodological proposal as an alternative for development in agribusiness, through Bio and nanotechnological processing of products with industrial value.

**Germination, Nanoparticle, Promoter, Agricultural**

**Resumen**

Las nanopartículas (NP) han atraído mucha atención debido a sus aplicaciones potenciales en biología, celdas solares y dispositivos optoelectrónicos que incluyen cosmética, ciencia biomédica, dispositivos ópticos, genes de fármacos (Manesh *et al*.,2010). A diferencia de otras opciones de síntesis en la obtención de nanopartículas por Química Verde se utiliza un extracto vegetal como agente reductor, muchas veces dicho extracto puede provenir de residuos vegetales denominados lignocelulósicos. Investigaciones recientes han evidenciado la relación entre el desarrollo vegetal y los materiales nanoestructurados identificando que cierto número de ellos pueden actuar como promotores de la germinación, del crecimiento de plántulas y el desarrollo de plantas completas posicionándose como una alternativa para mejorar la producción agrícola (Lopez, 2022). Por lo anterior, el objetivo del presente estudio es desarrollar una propuesta sustentable para la producción de Nanopartículas de plata (Np´s Ag) y su aplicación como promotoras de la germinación de Calabacita (*Cucurbita pepo*), maíz (*Zea mays*) y cebada (*Hordeum vulgare*) cultivos en los que su producción consumo e impacto económico son de gran importancia para el Estado de México; considerando esta propuesta metodológica como una alternativa de desarrollo en la agroindustria, a través del procesamiento Bio y nanotecnológico de productos con valor industrial.

**Germinación, Nanopartícula, Promotora, Agricultura**

**Citation:** VARGAS-SOLANO, Zaira, GRANADOS-OLVERA, Jorge Alberto, PÉREZ-LOREDO, María Guadalupe and RANGEL-RUIZ, Karelia Liliana. Silver Nanoparticles as Germination and Growth Promoters in Zucchini (*Cucurbita pepo*), Maize (*Zea mays*) and Barley (*Hordeum vulgare*). Journal-Agrarian and Natural Resource Economics. 2023. 7-13: 1-6

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## 1. Introduction

Most of the conventional methods used to produce nanoparticles have disadvantages such as: the use of toxic chemicals and waste generation, which can cause environmental contamination. (Iravani *et al*., 2014). Consequently, in recent years there has been a growing interest in synthesis methods considered ecological. As is the so-called Green Synthesis, which suggests the use of organisms that include bacteria, fungi, and plant extracts, which can reduce metal salts and allow the formation of nanoparticles with desirable size and morphology for various applications (Azmath *et al*., 2016; Bhambure *et al.*, 2009).

Studies have shown (Shalaby, 2016) that the presence of Nanomaterials in the Environment generates a response from the elements that make up the ecosystem as well as that particularly in plants exposed to nanoparticles there are variations in their morphological and physiological development depending on the type of particle and exposure levels (Zohra et.al., 2023).

Mendez *et al*. (2016) showed the beneficial effect of zinc oxide nanoparticles in chili peppers (*Capsicum annuum*) of crops from the north of our country, identifying ideal concentrations for the full development of the germination process, in the growth of the seedling and the growth of plants under greenhouse conditions. Thus, identifying this nanomaterial as a resource available for agro-industrial development (Bahwirth et.al., 2023).

Metal nanoparticles have great potential in different industries (Yokesh et.al., 2014; Boroumand et.al.,2015, Athanassiou et.al.,2018). Its participation in the field of medicine, the generation of broad-spectrum microbicides (Baishya et.al., 2012), in the degradation of pigments, in the production of superconductors, among others, has been successfully and clearly proven.

Research has recently been published that highlights the incorporation of nanostructured materials and products that contain them in agro-industrial applications (Lopez, 2022; Gonzalez y Fuentes, 2010), emphasizing their ability to replace fertilizers, growth promoters and crop enhancers, which have left a long list of harmful effects on the soil, water and even on the producers' health.

Therefore, it is urgent that the products used in the cultivation process within the agro-industry be diversified to reduce the impacts generated on the environment and the health of those who use them, without changing their capacity to enhance agricultural productivity.

Studies carried out on crops from the north of our country (Méndez et.al., 2016) have shown the capacity of nanostructured zinc oxide in formulation with silver nanoparticles, as potential promoters of germination and plant growth of a variety of chili peppers. Derived from this, the present work aims to demonstrate the capacity of silver nanoparticles (Np's Ag) as promoters of the germination and growth of plant species of importance in the Estado de México State for their consumption, production, and commercialization.

According to SECAMPO (2022) the Estado de México State is the 5th largest producer of Barley grain at National level, covering a cultivated area of 37,146 hectares, with a production volume of 89,121 tons, in the case of Corn grain we are the 6th largest producer with a planted area of 488,337 hectares exceeding 1.5 million tons per year, additionally, zucchini is a vegetable of which we are the 9th largest producer nationwide with a cultivated area of almost a thousand hectares and a production of more than 13,000 tons per year. Therefore, research evidence applicable to the three crops mentioned here is of great importance for the Estado de México State's countryside and its producers.

The study of the use of Silver Nanoparticles as a promoter of germination and growth of zucchini, corn, and barley, seeks to set a precedent to establish an alternative to replace agrochemicals focused on increasing the yield and productivity of crops of commercial interest at small and large scale, within the area of influence of the Estado de México State and even at national level.

## 2. Methodology

### Green synthesis of silver nanoparticles

The synthesis of silver nanoparticles is carried out by green synthesis based on silver nitrate as a chemical precursor and the extract of the Mexican Marigold flower (*Tagetes erecta*) as a reducing agent (Figure 1).

**Figure 1** Mexican Marigold flower (*Tagetes erecta*) planted in the facilities of the UPCI.

A solution of silver nitrate $AgNO_3$ at a concentration of 2 mM is added with constant droplets to a volume of Mexican Marigold flower extract (*Tagetes erecta*) in a 500 ml flask with moderate shaking (Figure 2). Next, the solution is subjected to a sonication process for 40 min in an ultrasonic tank.

**Figure 2** Green Synthesis of Silver Nanoparticles

With this process, the formation of particle aggregates begins, which is manifested through a brown coloration (Figure 3).

**Figure 3** Silver Nanoparticles.

### Preparation of solutions of Ag Nanoparticles

Ranges of germination and plant growth promoting solutions were established with solutions of 500 µl -1000 µl/500ml, which were dispersed in sterile bidistilled water and placed in opaque containers for use in germination and plant growth tests.

### Evaluation of the ability to promote germination

The germination of zucchini (*Cucurbita pepo*), corn (*Zea mays*) and barley (*Hordeum vulgare*) seeds was carried out under greenhouse conditions, placing the seeds in a peat moss substrate. Zucchini, corn, and barley seeds were used to evaluate the germination of the plant species.

The procedure is based on the OECD test No. 208 with some modifications. A success indicator in test results is that in the control batch exposed to water, after the indicated time (96 hours), at least 70% of the Seeds must germinate, otherwise it should be replaced for another batch of seeds. These tests were performed under controlled laboratory conditions, in an atmospheric pressure incubator, at 25 ± 0.5 °C.

In each box, a layer of cotton was placed (Figure 4a) and on it 3 to 5 seeds of the species already mentioned, (Figure 4b) 1.5 mL of distilled water with a solution of Np's Ag are added at concentrations of 500, 750, 800, 900 and 1000 µl of silver nanoparticles solution in 500 ml of sterile bidistilled water (Figure 4c).

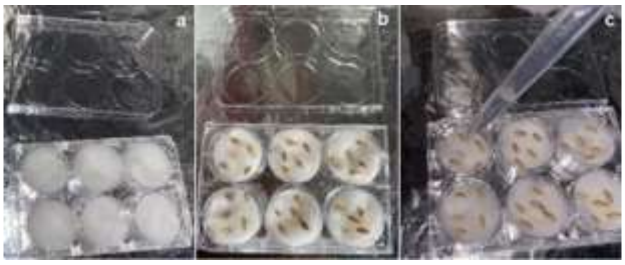


**Figure 4** Seed germination tests, a) Cotton placement, b) Seed count in each well, c) Addition of 1.5 mL of water / NP's Ag solution in each group of seeds

The test was carried out in quintuplicate, the samples must be kept, with a controlled temperature at 25°C for 96 hours.

## 3. Results

Adding the light-yellow flower extract to the silver nitrate solution produced the formation of Ag-Np's, which showed a color change due to the presence of surface plasmon resonance.

Figure 5 shows the UV-Vis spectrum of the silver nanoparticles synthesized by this route, which shows a similar behavior according to that reported by Sally D. Solomon (2007), the absorbance is between 395-430 nm attributable to a particle size between 15-30 nm.

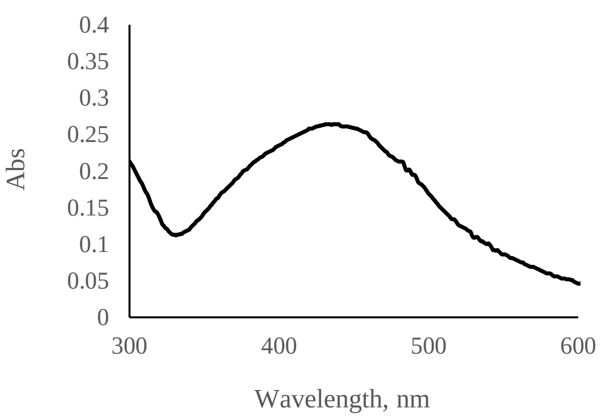


**Figure 5** UV-Vis spectrum of the $AgNO_3$-Mexican Marigold flower solution

### Germination evaluation with Np's

The use of Np's as germination and growth promoters of corn (*Zea mayz*), barley (*Hordeum vulgare*) and Zucchini (*Cucurbita pepo*) maximizes germination times and gives a better yield in terms of obtaining the harvest of plants from these seeds. Within the results obtained, it was observed that there is a better germination with respect to the plants that do not contain the silver nanoparticle (Table 1), although the control seeds that were sown had significant growth, it also had lower root density.

| Seeds | Germination % | Dissolution rate selected | Growth | |
|---|---|---|---|---|
| | | | Hight | Depth |
| Maíz H. tuxpeño norteño (*Zea mays*) Lote 1 | 76% | 800µl/500 ml | 11.3 cm | 21 cm |
| | | 950µl /500ml | 21 cm | 19 cm |
| | | 1000µl/500ml | 13.5 cm | 18 cm |
| Maíz H. zapalote grande, (*Zea mays L.*) Lote 2 | 60% | 800µl /500ml | 12 cm | 20 cm |
| | | 950µl/500ml | S/C | 18.5 cm |
| | | 1000µl/500ml | 14 cm | 18 cm |
| Maíz H. ratón, (*Zea mays amiláceo*) Lote 3 | 96% | 800µl/500ml | 14 cm | 21.5 cm |
| | | 950µl/500ml | 14 cm | 20 cm |
| | | 1000µl/500ml | 13.5 cm | 19 cm |
| Maíz H. cónico, (*Zea mays L.*) Breman p Lote 4 | 72% | 800µl/500ml | 13 cm | 17.5 cm |
| | | 950µl/500ml | 18 cm | 20 cm |
| | | 1000µl/500ml | 15 cm | 21 cm |
| Cebada (*Hordeum vulgare*) | 50% | 800µl/500ml | 13.3 cm | 16 cm |
| | | 950µl/500ml | 2.5 cm | 17.3 cm |
| | | 1000µl/500ml | 17 cm | 18.5 cm |
| Calabaza (*Cucurbita pepo*) | 10.52% | 800µl/500ml | 4.5 cm | 1 cm |
| | | 950µl/500ml | S/C | S/C |
| | | 1000µl/500ml | S/C | S/C |
| Control Maíz | 73.33% | Agua bidestilada sin Np's Ag | 14.5 cm | 21.5 |
| Control cebada | 73.33% | Agubidestilada sin Np's Ag | 5 cm | 16 cm |
| Control Calabaza | 73.33% | Agua bidestilada sin Np's Ag | 6 cm | 8 cm |

**Table 1** Evaluation of germination with nanoparticles.

The NPs effect begins to manifest itself from the germination of the seeds, reflecting in a greater emergence and uniformity that is observed in the final germination (Figures 6 and 7), mainly due to the penetration of nanomaterials in the seed, which allow increasing the imbibition of water and micronutrients, accelerating the reserves' degradation, and benefiting the first stages of the germinative process.

ISSN 2524-2091

**Figure 6** Germination test. Germination of corn (*Zea mays*) variety tuxpeño norteño, zapalote corn, mouse corn and cone corn) in concentrations of 500µl, 650µl, 850µl, 950µl 1000µl /500ml of bidistilled water, plumule growth and mesocotyl nodes

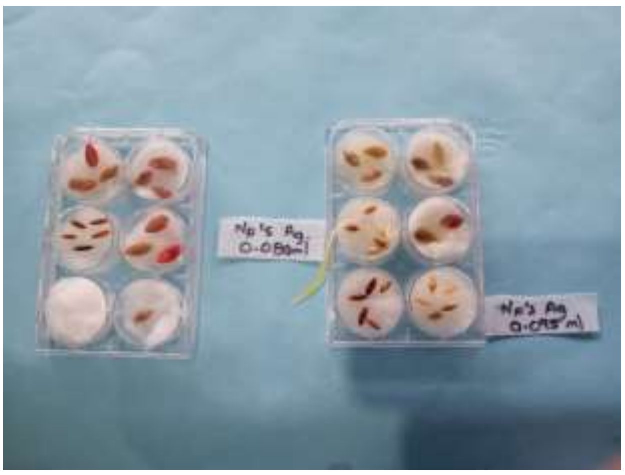

**Figure 7** Germination of barley (*Hordeum vulgare*) and zucchini (*Cucurbita pepo*) in concentrations 0.50ml, 0.65 ml, 0.80ml, 0.95ml, 1.0 ml/500ml of bidistilled water

The use of silver nanoparticles is also implemented to prevent microbial growth, especially for in vitro contamination and the appearance of pests in the seedling. Its application in different crops has increased seedling germination and growth, physiological activity, gene expression, and the biochemical activity of proteins and enzymes in plants, indicating its potential to sustainably improve the yield of agricultural crops.

## 4. Conclusions

Based on the obtained results, the UV-vis spectrum confirms the formation of Ag Np's by the methodology proposed by green synthesis using the Mexican Marigold extract.

The concentrations of NP's Ag that are considered germination promoters and/or growth promoters of at least one of the three proposed crops were identified, determining that the concentration of 800µl/500 ml of silver nanoparticles solution and water is the one that carries out more accelerated germination processes in conditions of roots growth and seedlings in accelerated conditions.

The use of silver nanoparticles serves as germination promoter and accelerates the growth of seedlings, which in this case were corn (*Zea mays*), zucchini (Cucurbita *pepo*) and barley (*Hordeum vulgare*), in addition to being effective in preventing microbial growth. Mainly these help the germination process, maximizing the germination times, given the fact that under normal conditions these seeds would take 7-10 days just to germinate.

Once the solutions concentrations of silver nanoparticles that have contributed to reducing germination times and promoting seedling growth have been identified, it is necessary to carry out bioaccumulation studies of these nanoparticles in vegetal tissues, monitoring their levels during plants growth and in the fruit and grains.

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ISSN 2524-2091