



LUCAS DE AZEVEDO SALES

**GREEN SYNTHESIS OF SILVER NANOPARTICLES USING
FLOWER EXTRACT**

**LAVRAS-MG
2024**

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Agronomy/Crop Science, for the degree of Master.

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SÍNTESE VERDE DE NANOPARTÍCULAS DE PRATA USANDO EXTRATOS DE FLORES

Thesis presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Agronomy/Crop Science, for the degree of Master.

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" Nothing in life is to be feared, only understood. Now is the time to understand more, to fear less."

Marie Curie

RESUMO

A nanotecnologia permite a transformação de materiais macroscópicos em escala nanométrica, preservando suas propriedades químicas e potencializando sua reatividade, graças ao expressivo aumento da área de superfície e às características únicas adquiridas nessa escala. Essas características fazem da nanotecnologia uma ferramenta essencial para o desenvolvimento de aplicações sustentáveis e inovadoras na agricultura, como a ação antimicrobiana de algumas nanopartículas. Um exemplo notável são as nanopartículas de prata, que demonstram elevado potencial para promover melhorias na agricultura, oferecendo perspectivas promissoras para o manejo sustentável de patógenos e a otimização da produção agrícola. Porém, a síntese convencional de nanopartículas pode causar impactos ambientais devido aos compostos químicos utilizados no processo. Já a síntese verde utiliza substâncias naturais para a produção das nanopartículas, minimizando os danos causados ao meio ambiente. A síntese verde, utilizando extratos de flores, apresenta vantagens como menor impacto ambiental, além de ser um processo mais rápido e simples. Diante disso, este trabalho teve o objetivo de explorar a eficiência da síntese de nanopartículas de prata utilizando extrato de flor de *Dahlia pinnata*, bem como avaliar os efeitos antimicrobianos apresentados por essas nanopartículas, avaliando suas propriedades e seu potencial uso na agricultura. A eficácia da síntese foi avaliada por meio de testes laboratoriais que puderam dimensionar o tamanho das nanopartículas de acordo com fontes comerciais e da literatura. Esses testes consistiram no uso dos equipamentos Zeta-sizer e UV-visível, e os resultados obtidos foram significantes para a continuação do trabalho. Para observar a eficácia dos efeitos antimicrobianos dos compostos testados, foram realizados testes precisos em laboratório, nos quais foi possível observar detalhadamente o crescimento dos patógenos em placas de Petri contendo os compostos incorporados aos meios de cultura, demonstrando a eficácia no controle dos patógenos com as nanopartículas de prata.

Palavras-chave: Extratos vegetais. Nanotecnologia. Ação antimicrobiana.

ABSTRACT

Nanotechnology makes it possible to transform macroscopic materials on a nanometric scale, preserving their chemical properties and enhancing their reactivity, thanks to the significant increase in surface area and the unique characteristics acquired on this scale. These characteristics make nanotechnology an essential tool for developing sustainable and innovative applications in agriculture, such as the antimicrobial action of some nanoparticles. One notable example is silver nanoparticles, which have shown great potential for promoting improvements in agriculture, offering promising prospects for the sustainable management of pathogens and the optimization of agricultural production. However, the conventional synthesis of nanoparticles can cause environmental impacts due to the chemical compounds used in the process. Green synthesis, on the other hand, uses natural substances to produce nanoparticles, minimizing the damage caused to the environment. Green synthesis, using flower extracts, has advantages such as less environmental impact, as well as being a faster and simpler process. The aim of this study was to explore the efficiency of silver nanoparticle synthesis using *Dahlia pinnata* flower extract, as well as to evaluate the antimicrobial effects of these nanoparticles, assessing their properties and their potential use in agriculture. The effectiveness of the synthesis was assessed by means of laboratory tests that were able to size the nanoparticles according to commercial and literature sources. These tests consisted of the use of Zeta-sizer and UV-visible equipment, and the results obtained were significant for the continuation of the work. To observe the effectiveness of the antimicrobial effects of the compounds tested, precise tests were carried out in the laboratory, in which it was possible to observe in detail the growth of the pathogens in Petri dishes containing the compounds incorporated into the culture media, demonstrating the effectiveness of the control of pathogens with the silver nanoparticles.

Keywords: Leaf extracts. Nanotechnology. Antimicrobial action.

INDICADORES DE IMPACTO

A síntese verde desempenha um papel fundamental no desenvolvimento sustentável, especialmente na produção ambientalmente amigável de nanopartículas. A pesquisa intitulada "Síntese verde de nanopartículas de prata utilizando extrato de flores" tem por objetivo quantificar e caracterizar os impactos tecnológicos, ambientais e econômicos decorrentes da aplicação dessa tecnologia sustentável. Em contraste com os métodos químicos convencionais, a síntese verde utiliza recursos naturais e minimiza o uso de reagentes tóxicos, contribuindo para a redução dos impactos ambientais. Além disso, a produção de nanopartículas de prata com propriedades antimicrobianas tem grande potencial para o controle de patógenos na agricultura, tornando o manejo de doenças mais eficiente. O estudo se insere na quinta área temática da Política Nacional de Extensão, Meio Ambiente, ao propor soluções inovadoras e ambientalmente corretas para o setor agrícola. A pesquisa também está alinhada aos Objetivos de Desenvolvimento Sustentável (ODS), especialmente aos ODS 12 (Consumo e Produção Responsáveis) e ODS 15 (Vida Terrestre), por incentivar a adoção de práticas menos prejudiciais ao meio ambiente e o uso racional de insumos na produção agrícola. O caráter extensionista da pesquisa é evidenciado pela interação entre a Universidade Federal de Lavras (UFLA) e diferentes setores da sociedade, como o Horto Botânico da UFLA, o Laboratório de Materiais e Biosistemas e o Laboratório de Patologia Florestal, bem como pela internacionalização em conjunto com a The University of Georgia (Georgia, EUA), onde foi possível apresentar a tecnologia trabalhada e possibilitar futuras aplicações, colaborando para o desenvolvimento e aplicação das nanopartículas. Além disso, há uma clara transferência de tecnologia para produtores agrícolas, especialmente no combate a patógenos, resultando em benefícios diretos para o aumento da produtividade e da sustentabilidade das lavouras. Os impactos tecnológicos são amplamente reconhecidos, visto que a aplicação das nanopartículas de prata pode revolucionar o controle fitossanitário, reduzindo a dependência de agroquímicos tradicionais que causam impactos ambientais negativos. Do ponto de vista econômico, a adoção dessa tecnologia tem o potencial de aumentar significativamente a produtividade agrícola, reduzindo as perdas causadas por doenças e, conseqüentemente, melhorando a eficiência dos sistemas de produção. Adicionalmente, o conhecimento gerado contribui para a formação acadêmica e técnica de estudantes e profissionais envolvidos, fortalecendo a pesquisa científica e a transferência de inovações para o setor produtivo. Dessa forma, a pesquisa se configura como um exemplo de alinhamento entre ciência, sustentabilidade e inovação tecnológica, promovendo impactos positivos tanto no ambiente acadêmico quanto no setor agrícola, em conformidade com as metas globais de desenvolvimento sustentável.

IMPACT INDICATORS

Green synthesis plays a key role in sustainable development, especially in the environmentally friendly production of nanoparticles. The research entitled “Green synthesis of silver nanoparticles using flower extract” aims to quantify and characterize the technological, environmental and economic impacts resulting from the application of this sustainable technology. In contrast to conventional chemical methods, green synthesis uses natural resources and minimizes the use of toxic reagents, helping to reduce environmental impacts. In addition, the production of silver nanoparticles with antimicrobial properties has great potential for controlling pathogens in agriculture, making disease management more efficient. The study falls within the fifth thematic area of the National Extension Policy, the Environment, by proposing innovative and environmentally friendly solutions for the agricultural sector. The research is also aligned with the Sustainable Development Goals (SDGs), especially SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land), by encouraging the adoption of practices that are less harmful to the environment and the rational use of inputs in agricultural production. The extension character of the research is evidenced by the interaction between the Federal University of Lavras (UFLA) and different sectors of society, such as the UFLA Botanical Garden, the Materials and Biosystems Laboratory and the Forest Pathology Laboratory, as well as the internationalization in conjunction with The University of Georgia (Georgia, USA), where it was possible to present the technology worked on and enable future applications, collaborating in the development and application of nanoparticles. In addition, there is a clear transfer of technology to agricultural producers, especially in the fight against pathogens, resulting in direct benefits for increased productivity and the sustainability of crops. The technological impacts are widely recognized, since the application of silver nanoparticles can revolutionize plant health control, reducing dependence on traditional agrochemicals that cause negative environmental impacts. From an economic point of view, the adoption of this technology has the potential to significantly increase agricultural productivity, reducing losses caused by diseases and, consequently, improving the efficiency of production systems. In addition, the knowledge generated contributes to the academic and technical training of the students and professionals involved, strengthening scientific research and the transfer of innovations to the production sector. In this way, the research is an example of alignment between science, sustainability and technological innovation, promoting positive impacts both in the academic environment and in the agricultural sector, in line with global sustainable development goals.

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1. INTRODUCTION

Nanoparticles are products derived from nanotechnology, which involves the manipulation and use of materials on a nanometric scale. They have significant relevance in technological advancement, being applicable in various areas due to their unique properties and innovative potential. Areas such as health, using materials that need to be sterile and of nanometric precision; materials engineering, developing products that are efficient and precise; areas such as agriculture, which is increasingly in need of products that are more efficient and more economical (KUMAR et al., 2020). Nanotechnology is explained as a process that separates, consolidates and deforms a given material at the level of a molecule or atom. In other words, the material on a macro scale will be exposed to processes that cause it to be reduced to a factor of 10^{-9} , thus becoming a nanomaterial (BAYDA et al., 2020).

There are also processes that consist of the reverse path, whereby smaller components such as atoms and ions can result in larger particles, bringing more precision to the size, composition and shape of the particles that will result from this process (BAIG et al., 2021).

Macromaterials that are transformed into nanomaterials undergo physical changes, while their chemical characteristics are preserved, allowing them to be used effectively. When the material is reduced to the nano scale, its contact surface becomes much more reactive and its properties become more evident, offering great advantages in various applications. (SOARES et al., 2017).

There are two main approaches to producing nanomaterials: top-down and bottom-up. The top-down strategy breaks down larger materials into nanometric particles, maintaining the original chemical composition. The bottom-up approach builds nanomaterials from atoms or molecules, allowing greater control over the size and shape of the resulting particles. These techniques are key to exploiting the unique properties of nanomaterials in various technological applications (ABID et al., 2022).

Knowing their potential and effectiveness, the use of nanoparticles is becoming increasingly common in various areas of activity, such as agriculture, due to the frequent search for solutions for food production in the world and for more sustainable agriculture. Today, the advance of technology in agriculture is remarkable, and the benefits and opportunities brought by these technologies, such as nanoparticles, are even more impressive (Lowry et al., 2019).

Nanoparticles perform several important functions, including their use in pesticides and fertilizers, where they improve the efficiency of these substances (NARS, 2019; KUMAR et al., 2022). In addition, nanoparticles have shown potential in improving plant germination, contributing to healthier and more vigorous growth (TIMOTEO et al., 2019).

A disadvantage related to the use of nanoparticles lies in their traditional synthesis, i.e. synthesis leaves a harmful chemical trail due to the compounds used in the process, which may end up being discarded and leaving harmful residues for the population and the environment. However, there is a process in which parts of plants are used to obtain the same products, which is called green synthesis (BANACH; PULIT-PROCIAK, 2016). In this process, unlike ordinary synthesis, parts of plants are used that have an abundance of the compounds needed to carry out green synthesis. This process is interesting for the reuse of waste from industries and can bring increasing sustainability to the synthesis of nanoparticles (Dhanalakshmi et al., 2024).

Thus, synthesis becomes less harmful to the environment and is often quicker and easier to carry out (BURLEC, A. F. et al., 2022). The green synthesis of nanoparticles, using biological agents such as plant extracts and microorganisms, offers an environmentally friendly approach to the production of nanomaterials (RAOTA, 2018). These nanoparticles improve the efficiency of fertilizers, promoting healthier plant growth and reducing environmental impacts. In addition, they have shown great effectiveness in combating agricultural pathogens such as fungi, bacteria and viruses, due to their antimicrobial properties (FATIMA; HASHIM; ANEES, 2021).

Notable examples include silver, copper and zinc nanoparticles, which protect crops in a sustainable way and help mitigate pathogen resistance to conventional treatments (ANSARI et al., 2023). The aim of this work was therefore to produce silver nanoparticles through the green synthesis process, using flowers from ornamental plants, and to test their antimicrobial action.

2. THEORETICAL FRAMEWORK

2.1 Nanotechnology

Nanotechnology basically consists of the process of separating, consolidating and deforming materials by a molecule or atom (KUMAR et al., 2020). Nanotechnology is an area of Nanoscience, which observes, measures, manipulates, controls and manufactures materials on the nanometer scale. The prefix “nano” refers to something very small, representing in the

metric system a factor of 10^{-9} , i.e. one millionth of a meter (BAYDA et al., 2020). Thus, Nanoscience studies structures and molecules that have dimensions in the range of 1 to 100 nanometers, and the technology that uses them in practical applications to develop materials and devices is called Nanotechnology (MANSOORI; SOELAIMAN, 2005).

Nanotechnology was introduced by the American physicist and Nobel Prize winner Richard Feynman, when in 1959 he gave a lecture at the end of the American Physical Society meeting at the California Institute of Technology (Caltech) entitled “There's Plenty of Room at the Bottom” (BAYDA et al., 2020). In this lecture, Richard makes clear his idea that the manipulation and control of matter on a small scale could open up a huge range of possible properties when compared to macrostructured particles (FEYNMAN, 2011).

By realizing Feynman's ideas, he came to be considered the father of modern nanotechnology (BAYDA et al., 2020). Fifteen years after Feynman's lecture, the concept/term “Nanotechnology” was used by Japanese scientist Norio Taniguchi in the publication of an article entitled “On the Basic Concept of ‘Nanotechnology’” which he defined as: “nanotechnology consists mainly of the processing of separation, consolidation and deformation of materials by an atom or a molecule” (TANIGUCHI et al., 1974; TONET; LEONEL, 2019).

Nanomaterials have altered physical and chemical properties, in which many of the laws of classical physics are unable to explain these systems, and therefore behave according to the laws of quantum physics (SOARES et al., 2017). When miniaturizing materials into nanoparticles, the chemical composition is maintained, but thermal, optical, magnetic and electrical properties are altered, for example, and chemical reactions can occur between different chemical elements in much smaller proportions (LEONEL, 2010). A factor that is related to the alteration of physical and chemical properties is the greater surface area of the nanomaterial, which is one of the great advantages of nanotechnology, because nanoparticles are much more reactive (LEONEL, 2010; SU et al., 2019).

Due to the unique physical and chemical properties of nanoparticles, their use and exploitation is carried out in various areas, such as chemistry, physics, biology, medicine, engineering, electronics and environmental issues (NASR et al., 2015; BAYDA et al., 2020). With the need to increase food production in the face of a growing world population, new technologies that combine sustainability and increased productivity are essential for agriculture

(LOWRY et al., 2019). Since they have several uses in agriculture, efficiency and sustainability, numerous patents and products involving nanoparticles have been developed (MAHAKHAM et al., 2017; VITHANAGE et al., 2017; TIMOTEO et al., 2019; PEREIRA et al., 2021).

2.2 Nanoparticles in Agriculture

Nanoparticles synthesized by green methods, using plant extracts, have shown positive effects on agricultural growth and productivity, especially in increasing nutrient absorption efficiency and plant resistance. Silver nanoparticles, for example, synthesized from plant extracts such as green tea and neem, have been applied to agricultural crops and have been shown to improve germination and initial plant development, with less dependence on conventional fertilizers (VANLALVENI et al., 2021). In addition, zinc nanoparticles, synthesized using green synthesis methods, have increased plant resistance to pathogens and promoted the growth of crops such as tomatoes and wheat, resulting in more vigorous and productive plants (HAMED; OBEID; ABU-HUWAIJ, 2023). Thus, these biogenic nanoparticles have great potential in the application of pesticides and fertilizers, being used in the formulation as carriers of the molecules of interest, optimizing the management of pests and diseases and allowing the use of encapsulated fertilizers for a slow and precise release of nutrients, increasing agricultural productivity (WANG et al., 2024).

Nanoparticles have various functions in plants due to their ability to penetrate the physiological system and their chemical properties. Among these functions are: improving water absorption efficiency, transferring DNA to plant tissues through a bombardment system, delivering herbicides stably to crops, activating the reproductive system and adapting the functions and expressions of genes (DELFANI et al., 2014; RICO et al., 2014; LAHIANI et al., 2015; KALE et al., 2021). Another important functionality of nanoparticles is the efficient transport of nutrients, pesticides and fertilizers from the root to the aerial part of plants (YEARLA; PADMASREE, 2016).

In addition, the chemical form in which some nutrients, such as nitrogen (N) and phosphorus (P), are made available has a low efficiency of use by the plant, which results in an excessive application of fertilizers and the release of unused fertilizers into the environment (KUMAR et al., 2022). The use efficiency of nitrogen fertilizers (urea) is approximately 30-40%, while that of phosphate is around 20-40% in most agricultural environments (DUAN et al., 2016). Because their diameter is smaller than the pores in the plant cell wall, nanoparticles

can penetrate membranes and act as carriers for nutrients and pesticides (LOWRY et al., 2019). This makes it possible to control the mobility of nutrients and pesticides, allowing them to be supplied in a controlled manner, without excesses (NARS, 2019; KUMAR et al., 2022).

Due to their antimicrobial activities, nanoparticles are also effective in reducing or delaying the incidence of fungi and bacteria, preserving the quality of fruit and vegetables for longer and reducing post-harvest losses. These properties make nanoparticles useful both in preserving and extending the shelf life of fresh produce and in active packaging, which reduces the respiration and transpiration of stored food.

In the context of nanoparticles in agriculture, the role of silver nanoparticles stands out. They have been widely studied and used in various applications, playing an important role in scientific discoveries about the use of these technologies in the field.

2.3 Silver Nanoparticles

Silver nanoparticles have been widely studied and used due to their unique properties and versatility in various applications and are the most widely used nanoparticle in industry (VANCE et al., 2015). Silver nanoparticles have high electrical conductivity, high reduction power, high photoelectric and catalytic activities and interesting optical and magnetic properties, making them more efficient than silver on a macro and micro scale (KUMAR et al., 2017; KALE et al., 2021). In addition, they are recognized for their antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory properties (KALE et al., 2021).

In the health sector, silver nanoparticles have been explored for the development of antibacterial materials, such as dressings and coatings for hospital surfaces, due to their effectiveness in eliminating pathogenic microorganisms. In addition, silver nanoparticles show promising potential in the treatment of diseases such as cancer, through photothermal therapies and controlled drug release (GOMES et al., 2021; SOFI et al., 2022). The antimicrobial efficacy of AgNPs is attributed to their ability to release silver ions (Ag^+), which are highly reactive and can cause damage to various cell structures and molecules within microorganisms. These ions can interact with cell membranes, causing their destabilization and increased permeability, thus leading to cell death. In addition, silver ions can penetrate cells and interact with DNA, proteins and other essential components, inhibiting cell replication and metabolic functions (CARREIRA et al., 2009).

In the textile sector, silver nanoparticles are widely used to confer antimicrobial and odorizing properties to fabrics, making them more hygienic and durable. These properties are very useful in applications such as sportswear and hospital uniforms (ZHOU et al., 2018; ABU-QDAIS et al., 2021).

In agriculture, silver nanoparticles (AgNPs) have been used to promote plant development and protection, as well as for food packaging. Studies have shown that AgNPs have effective antimicrobial properties against a wide range of microorganisms, including bacteria, fungi, yeasts and viruses (KALE et al., 2021). This is particularly relevant in the context of the phytopathogenic microorganisms *Calonectria* sp., *Ceratocystis* sp., *Xanthomonas* sp. and *Ralstonia* sp., which are responsible for costly losses in forestry and agricultural production (CARUSO et al., 2005; INDRAYADI et al., 2023; LI et al., 2023; RYAN et al., 2011).

AgNPs differ from conventional antibiotics in that they do not cause resistance in bacteria. This is because they do not act on a single site, but on several levels, such as the bacterial wall, blocking electron transfer, cellular respiration and replication, due to the damage caused to proteins, RNA and DNA (RAJJOU et al., 2012). These multiple forms of action make AgNPs powerful tools in the control of agricultural pathogens, helping to mitigate the economic impacts caused by phytopathogenic diseases and contributing to the sustainability and efficiency of agricultural practices.

The phytopathogens *Calonectria* sp., *Xanthomonas* sp. and *Ralstonia* sp. cause significant economic losses in agricultural and forestry crops due to their ability to infect and destroy various species of cultivated plants. *Calonectria* sp. mainly affects Eucalyptus plantations, resulting in considerable economic losses due to reduced wood quality and plant mortality (LI et al., 2023).

Xanthomonas sp. affects a wide range of agricultural crops, including rice, citrus and tomatoes, leading to substantial production losses. Examples of diseases caused by *Xanthomonas* include bacterial spot in tomato (*Xanthomonas campestris* pv. *vesicatoria*), citrus canker in citrus (*Xanthomonas axonopodis* pv. *citri*) and bacterial spot in rice (*Xanthomonas oryzae* pv. *oryzae*), all characterized by rapid spread and difficulty in management due to the pathogens' resistance and ability to infect multiple hosts (Ryan et al., 2011).

Ralstonia solanacearum is the causative agent of bacterial wilt, which affects crops such as potatoes, tomatoes and bananas. This disease results in serious economic losses due to the pathogen's ability to survive for a long time in the soil and infect plants systemically. Examples of diseases caused by *Ralstonia solanacearum* include potato bacterial wilt, which can destroy entire plantations, tomato bacterial wilt, which leads to plant collapse, and banana bacterial wilt, which causes plants to soften and die (Caruso et al., 2005).

The toxicity of silver nanoparticles is related to their size, concentration, stability and the species in question (TIMOTEO et al., 2018). Thus, at certain concentrations, it is possible to obtain a more effective and rapid antimicrobial effect (MAHAKHAM et al., 2017). The application of silver nanoparticles in in vitro culture can also result in positive induction in root length and number of shoots, with no toxicity at certain concentrations (TIMOTEO et al., 2018; TIMOTEO et al., 2019a; TIMOTEO et al., 2019b).

The synthesis of silver nanoparticles can be carried out using physical, chemical and biological methods, but due to the requirement for extreme conditions and the use of chemical compounds that can be toxic and cause risks to the environment when referring to physical and chemical methods, the use of biological methods is preferred (KALE et al., 2021). Therefore, green synthesis has stood out as a more sustainable and safer alternative. Involving the use of plant extracts, microorganisms or non-toxic solvents as reducing and stabilizing agents to produce silver nanoparticles, reducing environmental impact and health risks (AL-AWADY et al., 2019; AL-KHATTAF et al., 2023).

2.4 Green synthesis

Green synthesis is a process in which a given nanoparticle is obtained through processes using substances of natural origin. This process is simpler, safer, cheaper and easier to carry out than chemical synthesis, which uses toxic chemicals (KATTA; DUBEY, 2021). Various plant extracts can be used in green synthesis, as long as they contain compounds with reducing and stabilizing activity, such as: anthocyanins, organic acids, flavonoids, terpenoids, tannins and phenolic compounds (KUMAR et al., 2020). Most of the compounds needed for the green synthesis of nanoparticles are found in fruit and ornamental species, such as grapes (*Vitis vinifera*), butterfly beans (*Clitoria ternatea*), dahlia (*Dahlia pinnata*), strawberries (*Fragaria ananassa*), among others (GHODAKE et al., 2010). The great advantage of this synthesis is

that waste biological materials from industry can be used, i.e. materials that are discarded by industries can be used for the synthesis of nanoparticles (RAOTA, 2018).

The use of flowers in the green synthesis process has become increasingly common, as they have more favorable and advantageous characteristics in terms of their antibacterial properties. Silver nanoparticles derived from flowers have a great antibacterial effect, so that they can cross the membrane of bacteria, directly interfering with the functioning of the cell (KUMAR et al., 2020).

The process of green synthesis of silver nanoparticles first involves the reduction of Ag⁺ ions to Ag⁰, using the bioactive compounds previously extracted from the plants. This is followed by the growth of the nanoparticles, which are then stabilized by substances present in the extract itself. The solution containing the silver nanoparticles is then used for various applications (ERENO, 2014; KUMAR et al., 2020).

Bioactive compounds extracted from plants must have bioreduction capacity and stability to be used in the synthesis of nanoparticles, and one species that has these characteristics is the dahlia flower (SARANGAPANY; MOHANTY, 2020), an ornamental and edible plant rich in anthocyanins and phenols (HERNÁNDEZ-EPIGMENIO et al., 2022). These compounds, present in the Dahlia flower, play a significant role in the process of bioreduction and stability of metal ions in metal oxide nanoparticles (PRABHU et al., 2022).

Another relevant plant species in this context is *Clitoria ternatea*, known as the butterfly bean, which also has bioactive compounds with reducing and stabilizing properties, such as anthocyanins and flavonoids, and has been explored for the synthesis of nanoparticles (RAUTELA et al., 2019). Although *Clitoria ternatea* shows significant potential for the green synthesis of nanoparticles, comparative studies indicate that Dahlia flower shows better results regarding the efficiency and stability of the nanoparticles produced, due to the higher concentration of phenolic compounds and anthocyanins (KUMAR et al., 2020; KUMAR et al., 2022).

Recent studies have shown that AgNPs synthesized by green methods exhibit antimicrobial activity comparable or even superior to nanoparticles produced by conventional chemical methods. The presence of organic compounds from natural reducing agents can contribute to the stability of AgNPs and enhance their antimicrobial activity (CARNEIRO et

al., 2007). In addition, green synthesis allows the incorporation of additional functionalities into AgNPs, such as the binding of specific molecules that can direct the nanoparticles to target microorganisms, increasing the efficiency of the antimicrobial treatment (ANKUDZE et al., 2024).

The practical application of AgNPs synthesized by green methods in products and technologies requires an in-depth understanding of their interaction with microorganisms and the environment. It is essential to assess the safety, efficacy and environmental impact of these nanoparticles throughout their life cycle. Continued research and the development of regulatory standards are crucial to ensure that the use of AgNPs contributes positively to human health and environmental sustainability, in line with the goals of nanotechnology.

3. METHODOLOGY

3.1 Preparation of Aqueous Extract

Firstly, the flowers of *Clitoria Ternatea* and *Dahlia pinnata* (Figure 1) located in the Universidade Federal de Lavras - UFLA Botanical Garden were harvested and were in full physiological development.

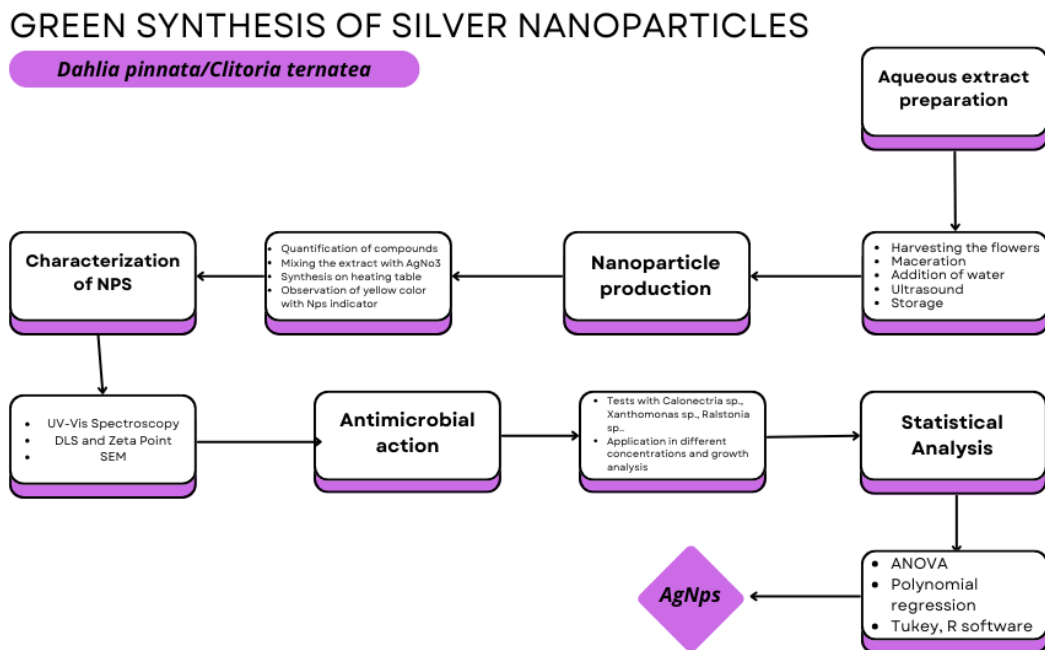
Figure 1 – *Clitoria ternatea* flower (A) and *Dahlia pinnata* flower (B)



Source: From author (2024)

The process of obtaining the aqueous extract from the petals (Figure 2) took place at the UFLA Post-Harvest Laboratory according to the method proposed by Franzen et al. (2018), with adaptations. 40 g of petals were macerated using liquid nitrogen and then 400 mL of distilled water was added. This solution was placed in an ultrasonic bath for 30 minutes and then filtered through filter paper. The aqueous extract generated was stored in glass containers (amber) with lids and taken to the fridge, which were then used as a reducing and stabilizing agent in the process of green synthesis of silver nanoparticles.

Figure 2 - Flowchart for determining the green synthesis of nanoparticles



Source: From author (2024).

The determination of antioxidant activity using the DPPH- (2,2-diphenyl-1-picrylhydrazyl) radical capture assay, which is based on the reduction of the DPPH- radical by antioxidants present in the extracts, was carried out as described by Brand-Williams et al. (1995) with adaptations. The reaction mixture consisted of 0.1 mL of plant extract and 3.9 mL of ethanolic DPPH solution (6.10⁻² mM). After 30 minutes of rest and under cover of light, the readings were taken on a microplate reader (EZ Read 2000) at 515 nm (BRAND-WILLIAMS et al., 1995). The ability to reduce the DPPH- radical (% reduction) was calculated using the following equation:

$$\% \text{ of reduction} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where:

A_{control}= Absorbance of the initial DPPH solution.

A_{sample}= Absorbance of the reaction mixture after 30 minutes.

3.2 Nanoparticles production

After the first stage of the experiment, production of the silver nanoparticle began using green synthesis. Various tests were carried out involving the two extracts, such as the quantification of anthocyanins, CFT (Total Phenolic Compounds) and B-carotene. Different concentrations of Silver Nitrate (0.01M and 0.001M) were used, as well as two different methods, which were using the hydrothermal and the other method using the “water bath” process (RAUTELA; RANI; DEBNATH (DAS), 2019) with hot oil on the heating table, where test tubes were placed with the solution and heated to 90 degrees for 20 minutes (MANI, et al., 2021). For the preparation of the nanoparticles, the green synthesis method using heating on a hot table was used, compared to the hydrothermal method. This procedure was chosen due to its greater speed and simplicity, as well as the fact that it requires a smaller quantity of compounds for synthesis. As shown in Figure 2, for the synthesis, the *Dahlia pinnata* extract and 0.01M AgNO₃ were mixed, and the solution was heated to 90°C for 20 minutes. The effectiveness of the synthesis was monitored by observing the color of the solution, which should have a yellowish hue characteristic of the formation of silver nanoparticles (Perveen et

al., 2021). After synthesis, the stability of the nanoparticles was assessed by observing the absence of precipitation over several days.

3.3 Characterization of Nanoparticles (UV-Vis, SEM, EDX, DLS, Zeta Potential)

Using the “water bath” process (RAUTELA; RANI; DEBNATH (DAS), 2019) to synthesize the Nps with the *Dahlia pinnata* extract, it was observed that the AgNp at 0.01M did not precipitate in the container, so it was decided to reproduce the entire synthesis process and take it to the UNIFAL Drug Delivery Systems Research Laboratory (LSLF), where these nanoparticles were measured using the Zetasizer equipment. The effectiveness of the synthesis was also observed in relation to time, where the AgNps were tested one day after synthesis and another test after 30 days of synthesis.

The characterization stage of the silver nanoparticles consisted of a series of analyses to evaluate their properties and guarantee the quality of the solution produced. Firstly, UV-Visible absorption spectroscopy was carried out to assess the concentration of the silver nanoparticle solution (ZOOK et al., 2011).

The hydrodynamic size of the silver nanoparticles was characterized by Dynamic Light Scattering (DLS), which provided information on the size distribution, polydispersity and aggregation of the particles, essential for assessing the stability of the nanoparticles in solution. In addition, using the same equipment (Malvern 3000 Zetasizer), the stability and electrical charge of the nanoparticles was determined using the ZETA Potential. The ZETA Potential test is important for measuring the repulsion force between particles, as it provides information about their dispersion in a solvent. ZETA Potential values close to zero indicate agglomeration and low stability, while high and positive values indicate stable dispersion (MALVERN, 2013).

The process of preparing the samples for scanning electron microscopy (SEM) was carried out meticulously, following a series of precise steps to guarantee the quality of the observations. Initially, the samples were placed in labeled microtubes, to which chemical components such as Karnovsky's solution (2.5% glutaraldehyde; 5% formaldehyde in 0.05M sodium cacodylate buffer; pH 7.2; 0.001M CaCl₂) were added to completely cover the samples. The samples were kept in this solution for 42 hours in a refrigerated environment (BOSSOLA et al., 1998).

After pre-fixation, the samples went through several subsequent steps. First, they were washed repeatedly in a 0.05M cacodylate buffer and then subjected to the addition of osmium tetroxide in another buffer. After this stage, the samples were washed with distilled water and dehydrated in acetone solutions at increasing concentrations (25%, 50%, 75%, 90% and 100%). Dehydration was followed by drying to the critical point. The samples were then transferred to special, labeled supports and subjected to a gold bath to increase their resistance and conductivity.

In the next phase, the samples were mounted on supports known as stubs, which can be made of carbon, brass or aluminum with a diameter of 12-13 mm. Carbon adhesives (a conductive material) were used to attach the samples to the stubs, which were duly identified. After mounting, the samples were bathed in gold and then viewed using a Clara scanning electron microscope (SEM).

Four sample stubs were mounted: two with Petri dish samples containing untreated culture medium and two with culture medium containing silver nanoparticles. Observations revealed a difference in the growth of the mycelia of the *Calonectria pteridis* fungus, as well as a difference in the structure of the mycelia, hyphae and conidia.

3.4 Antimicrobial Activity (Fungi and Bacteria)

During the study, we carried out tests to assess the antimicrobial efficacy of the silver nanoparticles. In collaboration with the Forest Pathology Laboratory at the Federal University of Lavras (UFLA), it was possible to carry out all the steps to confirm the antimicrobial activity of the synthesized nanoparticles.

- **Evaluation of the effect of nanoparticles on the growth of *Calonectria sp.* obtained from eucalyptus (*Eucalyptus globulus*).**

The culture medium BDA (Potato Dextrose Agar) was used for the growth of *Calonectria sp.* To analyze the three solutions: A- Dahlia Extract, B- Silver Nitrate and C- Nanoparticles, 6 treatments were applied, with a control and 5 concentrations for each solution: 0.1%, 0.2%, 0.3%, 0.4% and 0.5% per liter of growing medium. Discs with a diameter of 6mm were cut out using a plastic straw and then placed in the culture medium in the center of each 8cm diameter square petri dish (the dishes were previously plated, with the sides marked for evaluation). The material was then taken to a BOD (biochemical oxygen demand) incubator for

growth for ± 12 h at 25°C. On each axis, 4 growth measurements were taken per plate, analyzing growth using a digital caliper, the evaluations took place until the fungus had completely taken over the plates. Growth was assessed 24 hours after inoculation and repeated daily for 11 days until the mycelium had taken over the entire plate.

- **Evaluation of the effect of nanoparticles on the growth of *Xanthomonas sp.* and *Ralstonia sp.*, obtained from eucalyptus (*Eucalyptus globulus*).**

For the growth of *Xanthomonas sp.* and *Ralstonia sp.* bacteria, the 523-culture medium proposed by Kado and Heskett (1970) was used. For one liter of medium, 20 g of agar, 10 g of sucrose, 8 g of hydrolyzed casein, 4 g of yeast extract, 2 g of K₂HPO₄ and 0.3 g of MgSO₄ were used. To analyze the three solutions: A- Dahlia Extract, B- Silver Nitrate and C- Nanoparticles, 6 treatments were applied, with a control and 5 concentrations for each solution: 0.1%, 0.2%, 0.3%, 0.4% and 0.5% per liter of culture medium. The minimum bactericidal concentration (MBC) was determined by serial dilution, consisting of 4 dilutions of bacterial suspension. Two plates were used for each treatment of solutions A, B and C, each plate contained 4 dilutions of bacterial suspension, and each dilution was made with 4 drops (containing 10 μ l of bacterial suspension each drop), each drop being considered a repetition. Thus, each solution treatment (A, B and C) consisted of 8 repetitions (drops of bacterial suspension) for each dilution tested. After the microdrops were made, the plates were incubated in a bacteriological oven for 24 hours at a temperature of 35 ± 2 °C. Once the colonies were visible, the number of colonies formed was counted at the best dilution.

3.5 Statistical Analysis

The results were subjected to the Shapiro-Wilk test for normality of residuals and Bartlett's test for homogeneity of variances, and then analysis of variance (ANOVA) was carried out with the data transformed into $\sqrt{x+1}$. The effect of the doses was analyzed using polynomial regression, testing linear and quadratic models. The criteria for choosing the model were the significance of the F test ($p \leq 0.05$) and the highest coefficient of determination (R^2). The sources were analyzed within each dose using the Tukey test ($p \leq 0.05$). Statistical analyses were carried out using the R statistical program (R Core Team, 2024).

4. RESULTS

5.1 Characterization of the flower extract of *Dahlia pinnata* and *Clitoria ternatea*

Analyzing the characteristics of the extracts from the flowers of the ornamental plants *Dahlia pinnata* and *Clitoria ternatea*, it was possible to observe, according to Table 1, that the extract with the most favorable compounds for the production of the synthesis was the *Dahlia pinnata* extract, given that the values obtained for total phenolic compounds, anthocyanins and DPPH were higher than the values obtained in the *Clitoria ternatea* extract for the same compounds. The only value that *Clitoria ternatea* extract showed to be superior was Beta-carotene/linoleic acid, but there is no justification for using it.

Table 1 – Extract characterization values

Determinations	<i>Dahlia pinnata</i>	<i>Clitoria ternatea</i>
Total phenolic compounds (mg/100 mL extract)	200.26±2.19	160.99±1.18
Anthocyanins (mg/100 mL extract)	9.43±0.30	4.09±0.67
DPPH (% reduction)	52.98±4.18	17.57±4.95
β-carotene/Linoleic acid (% protection)	13.19±3.37	26.81±5.81

Source: From author (2024)

5.2 Characterization of AgNPs

After the sample readings were taken in the Zeta-sizer equipment, it was found that the 0.01M silver nanoparticle made with *Dahlia pinnata* extract was indeed very stable and had a size within the standard established in the literature (1 - 100 nm), where it had a size of 180.5 nm and a Pdl of 0.342.

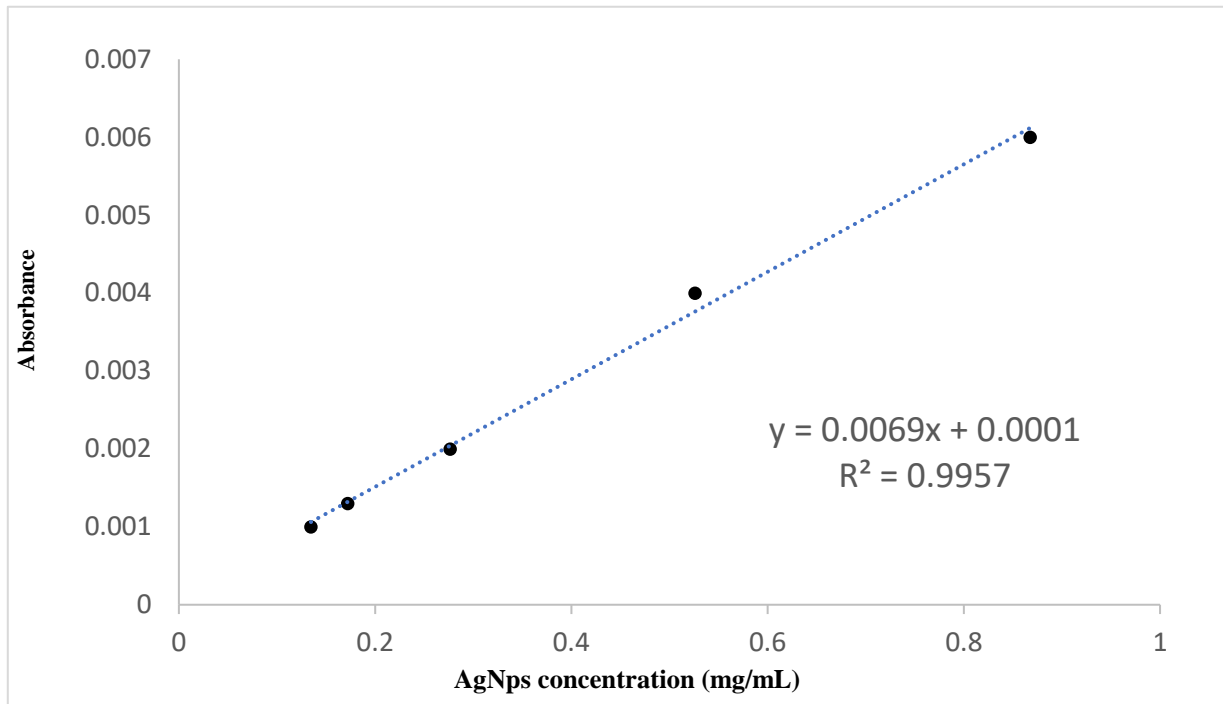
Table 2 – AgNP characteristics after different storage periods

Days	1°	30°
Z-Ave (d.nm)	142.9	133.8
Pdl	0.367	0.168

Source: From author (2024)

To determine the concentration of silver nanoparticles at 0.01M, a calibration curve was made using a silver nanoparticle standard. This calibration curve was obtained using ultraviolet-visible spectroscopy (UV-VIS, BEL Química) using a series of dilutions of the silver nanoparticle standard within the Lambert-Beer law (absorbance less than or equal to 1).

Graph 1 - Silver nanoparticle calibration curve



Source: From author (2024)

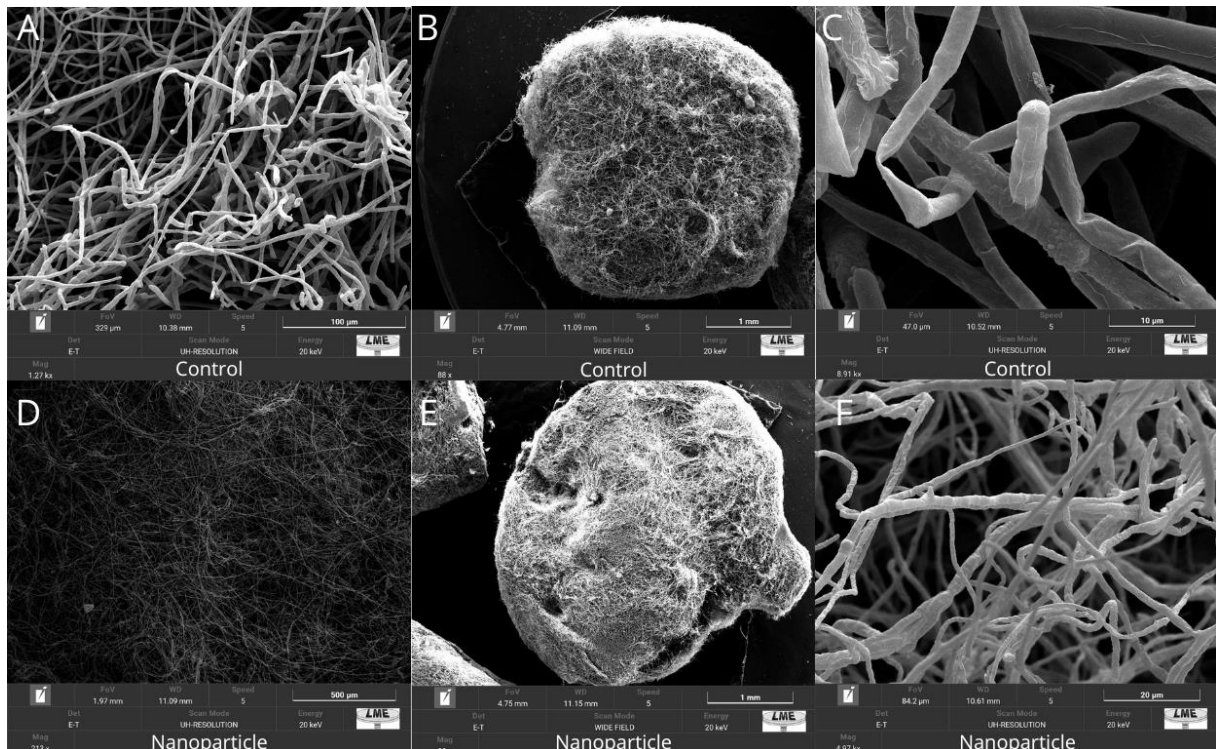
Graph 1 shows the concentration of silver nanoparticles on the x-axis (mg/mL) and the absorbance on the y-axis. The linear relationship between these two variables is described by the equation of the fitted line, which is:

$$Y=0,0069x+0,0001$$

Where Y is the absorbance and X is the concentration. The coefficient of determination $R^2=0.9957$ indicates an acceptable fit of the line to the data points, suggesting that the linear relationship explains the variation in absorbance well.

The statistical test used to verify the concentration of the 0.01M AgNps solution was the calculation of the coefficient of determination R^2 , which assesses the quality of the fit of the regression line to the experimental data. Using the analytical equation obtained, the concentration of the 0.01M silver nanoparticles was determined to be 0.04mg/mL.

Figure 3 – Effect of Silver Nanoparticles on *C.pteridis* sample



Source: From author (2024)

The observations showed significant differences in the growth and morphology of the mycelia of the *Calonectria amazonica* fungus. The images at the top of the figure show the control samples, where the structures of the fungus are more developed and uniform, highlighting the regularity and presence of conidia (FIGURE 3-A, 3-B, 3-C). In contrast, the images at the bottom, which represent the treatment with silver nanoparticles, reveal impaired mycelial development. The first image (FIGURE 3-D) illustrates the reduction in mycelial density compared to the control, indicating atypical growth and thin, incomplete mycelial structures. The second image (FIGURE 3-E) shows a thick 'layer' which is believed to be the effect of the nanoparticles, obstructing fungal development. Finally, the third image (FIGURE 3-F) highlights the lower thickness and density of the mycelial structures compared to the control.

As shown in Table 4 and in Graphs 2, 3 and 4, according to the statistical tests carried out in the R software, the data showed interesting results regarding the action of silver nanoparticles and silver nitrate against pathogens.

It was noted that the antimicrobial action of the extract treatment was not satisfactory, unlike the treatments containing AgNps and AgNo₃ (nanoparticles and silver nitrate).

According to the data, the bacteria only showed growth in the Extract treatment, thus demonstrating the effectiveness of the other treatments in controlling the pathogens.

In the case of the fungus (*Calonectria sp.*) the scenario was the same, the treatment containing *Dahlia pinnata* extract was not effective in controlling the pathogen, while the other treatments (AgNps and AgNo₃) were effective in controlling it and performed best at dose 2. The effect of the AgNps on the fungus can be seen in Figure 3, where it is possible to observe the controlling effect that is caused on the fungus.

Table 3. Summary of the analysis of variance for final growth (FC), mycelial growth velocity index (IVCM) and mycelial growth inhibition (PIC) of *Calonectria sp.* with application of different doses of extract, silver nitrate and nanoparticle.

Source of Variation	DF	Mean Square		
		CF	IVCM	PIC
Source (F)	2	3540.5 **	592.24 **	20208.2 **
Doses (D)	5	395.5 **	85.44 **	5070.7 **
Interaction (F x D)	10	150.0 **	24.56 **	847.7 **
Residual	72	1.2	0.49	15.8
C.V. (%)		4.3	9.6	10.7

ns = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$ by F-test

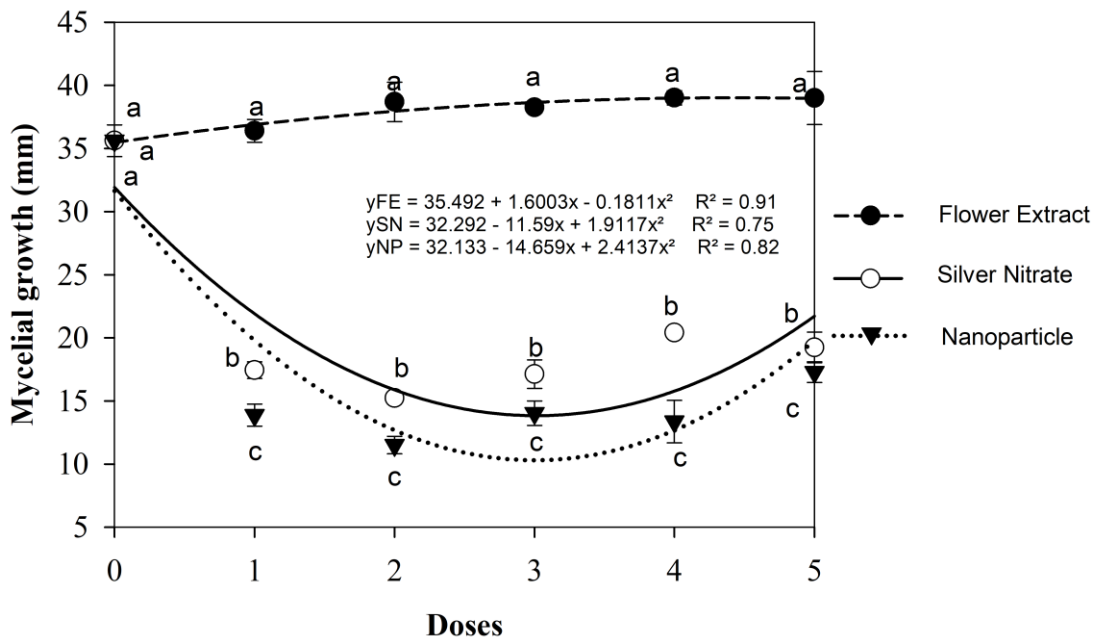
Table 4. Activity of different doses of extract, silver nitrate and nanoparticles on *Ralstonia sp.* and *Xanthomonas sp.* ● = Presence; ○ = Absence

Extract	% of each per litre				
	0,1%L-1	0,2%L-1	0,3%L-1	0,4%L-1	0,5%L-1
<i>Ralstonia sp.</i>	●	●	●	●	●
<i>Xanthomonas sp.</i>	●	●	●	●	●
Silver Nitrate	% of each per litre				

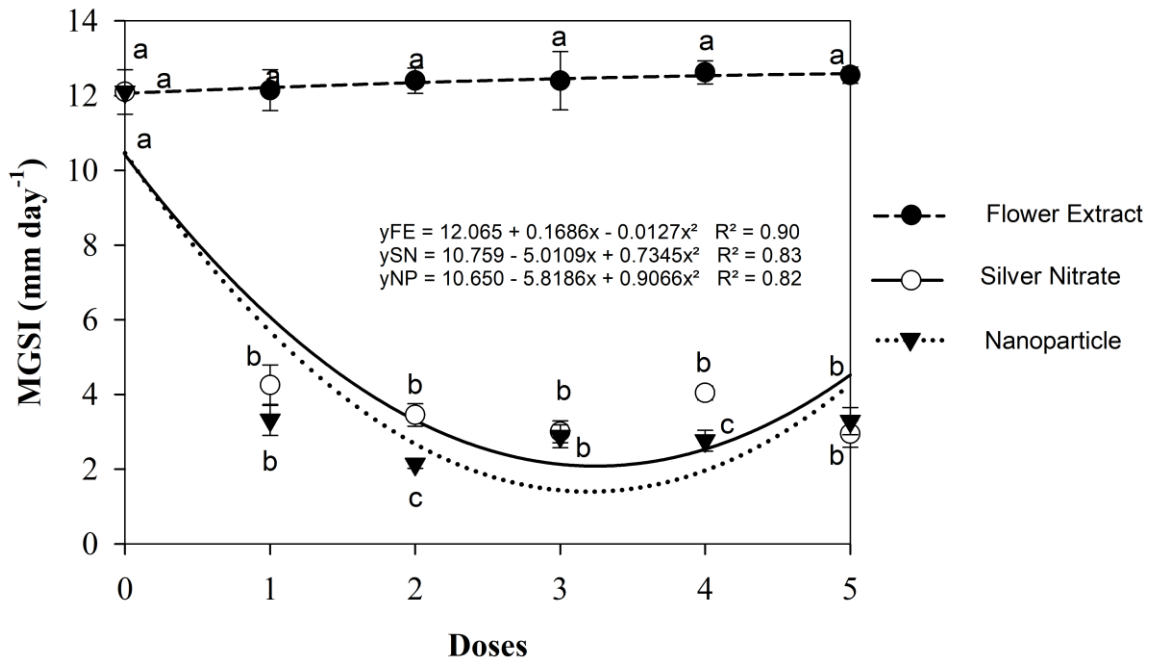
	0,05%L-1	0,1%L-1	0,15%L-1	0,2%L-1	0,25%L-1
<i>Ralstonia</i> sp.	○	○	○	○	○
<i>Xantomonas</i> sp.	○	○	○	○	○

Nanoparticle	% of each per litre				
	0,5%L-1	1%L-1	1,5%L-1	2%L-1	2,5%L-1
<i>Ralstonia</i> sp.	○	○	○	○	○
<i>Xantomonas</i> sp.	○	○	○	○	○

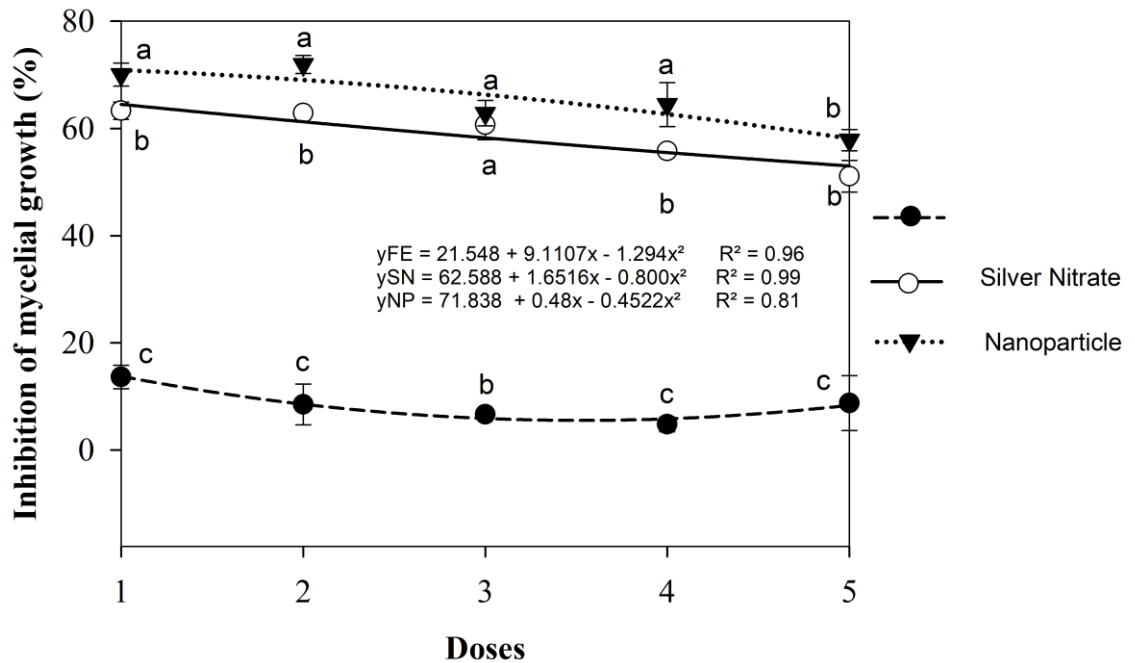
Control	% of each per litre				
	<i>Ralstonia</i> sp.	●	●	●	●
<i>Xantomonas</i> sp.	●	●	●	●	●



Graph 2 - Activity of different doses of extract, silver nitrate and nanoparticle on the mycelial growth of *Calonectria* sp. Similar letters in the same dose do not differ statistically by Tukey's test ($p \leq 0.05$).



Graph 3 - Activity of different doses of extract, silver nitrate and nanoparticle on the mycelial growth velocity index (IVCM) of *Calonectria sp.* Similar letters in the same dose do not differ statistically by Tukey's test ($p \leq 0.05$).



Graph 4 - Inhibition of mycelial growth of *Calonectria sp.* with application of different doses of extract, silver nitrate and nanoparticle. Similar letters in the same dose do not differ statistically by Tukey's test ($p \leq 0.05$).

5. DISCUSSION

The data was subjected to the Shapiro-Willk test for normality of residuals and Bartlett's test for homogeneity of variances, and then analysis of variance (ANOVA) was carried out with the data transformed into $\sqrt{x+1}$.

Table 1 shows the values for the characterization tests carried out on the extracts of the two ornamental plants, *Dahlia pinnata* and *Clitoria ternatea*. The extract characterization tests were expected to show which of the two would be more suitable for use in the synthesis of silver nanoparticles, i.e. which extract would have the best values for phenolic compounds, anthocyanins, reducing power and Beta-carotene. The importance of knowing these parameters in detail is that they are responsible for the best choice of extract to be used, bearing in mind that green synthesis is a more sustainable replacement for chemical synthesis, i.e. with green synthesis being efficient, chemistry becomes less used, leading to less waste in the environment (KATTA; DUBEY, 2021).

According to the tests carried out, it was notable that the extract from the flower of the ornamental plant *Dahlia pinnata* showed better results compared to the flowers of the ornamental plant *Clitoria ternatea*, since the values of almost all the tests were higher, with the exception of the value of the Beta-carotene test, in which the value presented by *C.ternatea* was higher than that presented by *Dahlia pinnata*, with no great difference in the interpretation of the data. The data obtained from the extracts corroborates the quantities of compounds needed to carry out the synthesis, given that the presence of anthocyanins, organic acids, phenolic compounds, flavonoids and tannins is necessary (KUMAR et al., 2020).

In relation to the data presented from the characterization carried out with the silver nanoparticle, it could be seen that the higher concentration (0.01 M) obtained a more satisfactory result than the lower concentration (0.001M). The concentrations used were based on syntheses carried out previously, where the behavior of the nanoparticles could be observed according to their respective precipitations.

According to the tests carried out on the Zetasizer at UNIFAL - MG, it was noted that the nanoparticles with a higher concentration showed greater stability, as well as their size being more satisfactory, remaining at around 180.5 nm and presenting a Pdl of 0.342. According to the literature, the size to be in the “nano” range would be 0 - 100 nm (BOHOLM; ARVIDSSON,

2016), however there is a margin of error which shows that this parameter can be considered up to 300nm, so the Nps are as expected (ZIEGLER; EYCHMULLER, 2011).

Scanning electron microscope (SEM) images showed that petri dishes inoculated with the fungus and containing nanoparticles incorporated into their culture medium had inefficient and even inhibited growth due to a kind of “glue” that the nanoparticles formed around the pathogen, preventing it from growing. This is an example of the antimicrobial action that silver nanoparticles have on fungi, opening discussion for more and more uses of these alternatives in agriculture (BAMAL et al., 2021).

The bacterial growth tests carried out with the extracts, silver nanoparticles and silver nitrate showed interesting and relevant results for practical application in pathogen control. Regarding the *Dahlia pinnata* extract, the results indicated that it was not significantly effective in inhibiting the growth of the bacteria tested. This can be attributed to the lower concentration of bioactive compounds with bactericidal action in the extracts, compared to the methods using AgNps and AgNo₃ (DURÁN et al., 2018).

Silver nanoparticles (AgNPs) have exhibited a potent antimicrobial action through various mechanisms that directly affect the integrity and functionality of bacterial cells. AgNPs adhere to the surface of the bacterial cell membrane, causing structural damage and increasing permeability. This results in rupture of the membrane and penetration of the nanoparticles into the cell, where they interact with organelles and biomolecules, causing significant dysfunction (MIKHAILOVA, 2020; MORE et al., 2023).

In addition, AgNPs induce the generation of reactive oxygen species (ROS), causing severe oxidative stress, which affects essential proteins and enzymes, inhibiting nucleic acid replication and ion transport processes (LOK et al., 2006; TIAN et al., 2007). The effectiveness of AgNPs is influenced by both the concentration and size of the nanoparticles, with smaller particles exhibiting greater antimicrobial activity due to the greater surface area for interaction with bacterial cells (RAFFI et al., 2010).

The second dilution was the minimum bactericidal concentration that allowed the colonies of *Ralstonia sp.* to be counted. However, it was not possible to count *Xanthomonas sp.* because all the dilutions failed to separate the colonies into distinct groups. Table 4 shows that the *Dahlia pinnata* extract solution favored the development and growth of both bacteria, with

no significant difference between treatments or compared to the control. On the other hand, the silver nitrate and nanoparticle solutions inhibited the growth of both bacteria, with no significant differences between the treatments, as shown in Figure 4.

Plant extracts have a diversity of compounds which, although they have bioactive properties, may not be in sufficient concentration to cause significant inhibition of bacterial growth. These results suggest that although ornamental plant extracts can be used in some applications, their effectiveness in bacterial control is limited when compared to more advanced methods, such as the use of silver nanoparticles (GONELIMALI et al., 2018).

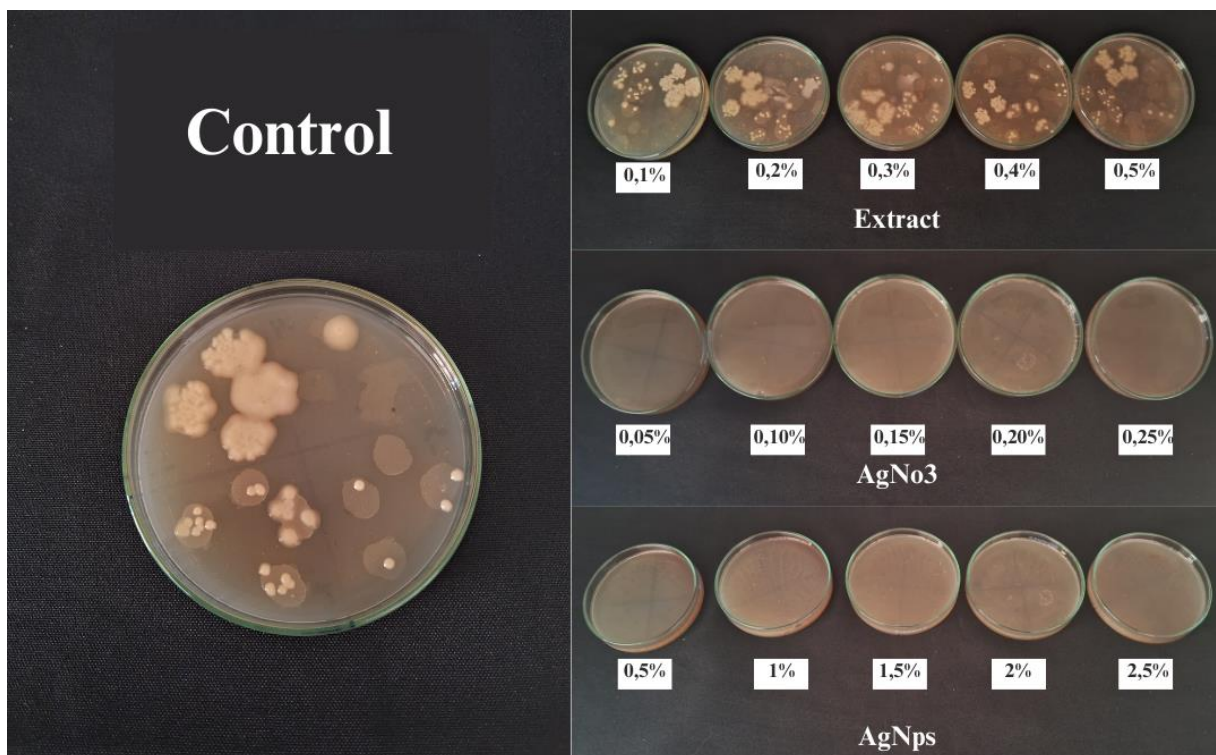


Figure 4 - Difference between treatments in samples of *Ralstonia sp.* **Control** - Petri dishes with conventional culture medium; **Extract** - Petri dishes with flower extracts (*Dahlia pinnata*); **AgNO₃** - Petri dishes with Silver Nitrate incorporated into the medium; **AgNps** - Petri dishes with Silver Nanoparticles incorporated into the medium.

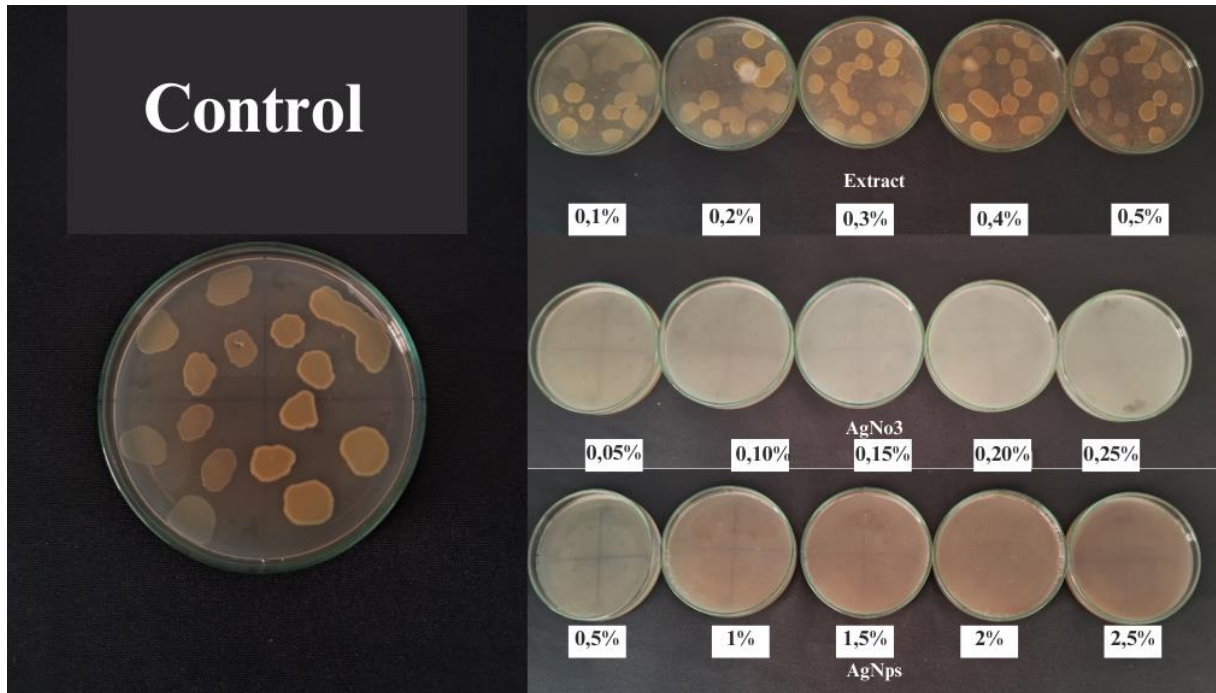


Figure 5 - Difference between treatments in samples of *Xanthomonas sp.* Control - Petri dishes with conventional culture medium; **Extract** - Petri dishes with flower extracts (*Dahlia pinnata*); **AgNO₃** - Petri dishes with Silver Nitrate incorporated into the medium; **AgNps** - Petri dishes with Silver Nanoparticles incorporated into the medium.

As shown in Graph 4, the results of the mycelial growth tests on the fungus *Calonectria sp.* showed that silver nanoparticles were more effective at inhibiting growth than plant extracts and silver nitrate. The graph data indicates that mycelial inhibition was significantly greater with the use of silver nanoparticles, reaching approximately 70%, while the silver nitrate solution showed inhibition that decreased slightly with increasing concentration, starting at around 60%.

The treatments shown in Graph 2 include *Dahlia pinnata* flower extract in 1, 2, 3, 4 and 5 ml solutions, 0.1M Silver Nitrate in 0.5, 1, 1.5, 2 and 2.5 ml solutions, and 0.01M Silver Nanoparticle in 5, 10, 15, 20 and 25 ml solutions. The data obtained revealed that the treatments with Silver Nitrate and Silver Nanoparticles showed significant differences compared to the control, while the treatment with *Dahlia pinnata* extract showed no significant differences.

As shown in Graph 2, during the test, all the doses of Silver Nanoparticle showed a significant difference, highlighting the effectiveness of this treatment. Concentration 2 of AgNPs (10 ml of 0.01M solution) showed the lowest mycelial growth, indicating greater

antimicrobial action against the fungus *Calonectria sp.* A significant difference can be observed in all doses of silver nanoparticles, thus highlighting their potential as an antimicrobial agent. These results underline the superiority of silver nanoparticles compared to plant extract and silver nitrate, especially in their ability to inhibit the growth of phytopathogenic fungi, reinforcing the importance of developing sustainable and effective methods for protecting agricultural crops.

Graph 3 shows the average mycelium growth rate (MGSI) in mm per day for three different treatments: *Dahlia* extract, silver nitrate and nanoparticle, using different concentrations of the treatments. The *Dahlia.sp* extract solution shows a slight increase in MGSI as the extract concentration increases but remains relatively stable at around 12 mm per day. The values do not differ significantly, as indicated by the letter “a” for all doses. In the Silver Nitrate solution, the MGSI decreases as the concentration increases, reaching its lowest point at dose 3 and then increasing again. The letters “b” and “c” indicate significant differences in the values compared to doses 1 and 5. While the Nanoparticle shows a similar pattern to the silver nitrate, with an initial reduction in MGSI up to dose 3, followed by an increase at doses 4 and 5. The letters “b” and “c” also indicate significant differences in the values compared to the other doses. The increase in mycelial growth may be due to the increase in concentration, which on the other hand indicates an increase in the amount of extract, which causes the fungus to resume development due to the greater presence of extract (MALIK et al., 2024; VANLALVENI et al., 2021).

In Graph 4, the treatments shown in the graph are flower extract (*Dahlia pinnata*) in solutions of 1; 2; 3; 4 and 5 ml, Silver Nitrate at 0.1M with solutions of 0.5; 1; 1.5; 2 and 2.5 ml and Silver Nanoparticle at 0.01M with solutions of 5; 10; 15; 20; 25 ml. According to the data obtained, the Silver Nitrate and Nanoparticle treatments showed significant differences, while the extract treatment did not. Considering the AgNps and AgNo₃ treatments, the concentration that showed the greatest inhibition of mycelial growth (greatest action of the treatments) was AgNps concentration 2, i.e. according to the data AgNps concentration 2 was responsible for inhibiting the mycelial growth of the fungus.

The significant impact of silver nanoparticles can be attributed to their ability to cause physical damage to the cell structures of the fungus, as well as inducing the production of reactive oxygen species that lead to cell death. The presence of nanoparticles around the

mycelium of the fungus, forming a kind of barrier or “glue”, also contributed to growth inhibition, showing the potential effectiveness of nanoparticles as antifungal agents (RODRIGUES et al., 2024).

6. CONCLUSION

This work has shown that the green synthesis of silver nanoparticles (AgNPs) using *Dahlia pinnata* flower extract is an efficient and sustainable approach, capable of minimizing the environmental impacts associated with conventional nanoparticle synthesis. The characterization of the AgNPs revealed that they have adequate size and stability, and are highly effective in inhibiting the growth of plant pathogens such as *Calonectria sp.*, *Xanthomonas sp.* and *Ralstonia sp.*

The results indicate that silver nanoparticles are effective due to their multiple mechanisms of action, including adhesion to the bacterial cell membrane, induction of reactive oxygen species (ROS) and penetration into bacterial cells, which results in the disruption of essential cellular functions. All the doses of silver nanoparticles tested showed significant differences in inhibiting the mycelial growth of *Calonectria sp.*, highlighting the effectiveness of this treatment.

In this way, the green synthesis of AgNPs using plant extracts not only provides a viable and environmentally friendly alternative to conventional synthesis but can also contribute significantly to the sustainability and efficiency of agricultural practices. It is hoped that this study will encourage the development and application of similar techniques for other nanoparticles and promote the adoption of more sustainable methods in agriculture and other areas. These findings reinforce the importance of exploring innovative and environmentally responsible solutions for the control of agricultural pathogens, contributing to the reduction of economic losses and the promotion of a more sustainable agriculture.

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