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Phytosynthesis of silver nanoparticles from the leaf extract of *Ocimum sanctum* (Holy Basil/Tulsi), their characterization, and incorporation into a biofungicidal biofertilizer developed from arecanut shell

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Abstract

Modern agriculture's heavy reliance on synthetic fertilizers and chemical fungicides has raised critical environmental concerns, underscoring the urgent need for sustainable alternatives. This study reports the green synthesis of silver nanoparticles (AgNPs) utilizing the aqueous leaf extract of *Ocimum sanctum* (Holy Basil), a medicinal plant renowned for its rich bioactive profile and antimicrobial efficacy. The plant extract served as an efficient, non-toxic reducing and stabilizing agent to synthesize AgNPs from a silver nitrate solution. Nanoparticle formation was initially indicated by a distinct color change in the reaction mixture and subsequently characterized via UV-Visible spectroscopy and Scanning Electron Microscopy (SEM) to determine their structural and physicochemical properties. To develop a value-added agricultural formulation, the synthesized AgNPs were uniformly incorporated into an organic biofertilizer matrix composed of composted arecanut shell waste and well-decomposed cow dung. The biofungicidal efficacy of this nanoparticle-enriched formulation was then evaluated against *Phytophthora capsici*, the devastating fungal pathogen responsible for foot rot disease in black pepper. By merging plant-mediated nanotechnology with the valorization of agricultural waste, this research offers a dual-action sustainable solution. The resulting formulation simultaneously provides essential crop nutrition and robust protection against fungal pathogens, demonstrating a viable, environmentally responsible alternative to conventional chemical inputs while adding economic value to agricultural waste.

Keywords: Phytosynthesis, silver nanoparticles, biofungicide, biofertilizer

1. Introduction

Agriculture remains the cornerstone of global food security and rural livelihoods (Godfray *et al.*, 2010)^[27]. To keep pace with a growing global population, modern farming has become deeply dependent on synthetic chemical fertilizers and pesticides (Tilman *et al.*, 2002)^[66]. While these inputs initially boosted crop yields, their excessive and prolonged application has severely degraded the agricultural matrix. Today, we face critical challenges including soil degradation, depleted beneficial microbial populations, groundwater contamination, and toxic chemical residues in food (Aktar *et al.*, 2009; Carvalho, 2017)^[1, 11]. Furthermore, plant pathogens are rapidly developing resistance to conventional treatments, underscoring the urgent need for eco friendly, sustainable, and cost effective alternatives (Pretty, 2008)^[50].

A promising path forward lies in integrating biofertilizers and biofungicides derived from renewable resources (Bhardwaj *et al.*, 2014)^[6]. Biofertilizers restore soil vitality by leveraging organic matter decomposition and microbial pathways to increase nutrient availability (Mahanty *et al.*, 2017)^[35]. Concurrently, biofungicides shield crops from pathogens using natural biocontrol agents or bioactive compounds instead of synthetic toxins (Choudhary and Johri, 2009)^[14].

An ideal substrate for these bio-inputs is agricultural waste, which promotes a circular farm economy (Bhat *et al.*, 2018)^[7]. In the southern states of India, arecanut cultivation generates massive quantities of arecanut shell waste (Jose *et al.*, 2021)^[29].

Typically burned or discarded which exacerbates local waste management issues and these shells are highly lignocellulosic and rich in organic carbon (Chowdappa *et al.*, 2014; Singh *et al.*, 2012) ^[15, 61]. Transforming this abundant biomass into a nutrient-rich compost offers a sustainable method for waste utilization while building a robust biofertilizer base (Zafar *et al.*, 2022) ^[71].

To elevate the functionality of these organic matrices, nanotechnology offers a revolutionary toolkit (Parisi *et al.*, 2015) ^[44]. Silver nanoparticles (AgNPs) are highly valued due to their exceptionally high surface area and broad spectrum antimicrobial activity against bacteria, viruses, and fungi (Rai *et al.*, 2009; Chhipa, 2017) ^[52, 12]. However, conventional chemical and physical synthesis methods often require hazardous stabilizing agents and high energy inputs, generating toxic by products (Iravani *et al.*, 2014; Rafique *et al.*, 2017) ^[28, 51]. This has driven a shift toward green synthesis or phytosynthesis (Mittal *et al.*, 2013) ^[40]. Plant extracts naturally abound with phytochemicals such as flavonoids, phenolics, terpenoids, and reducing sugars that act as non toxic, dual reducing and capping agents, making nanoparticle production rapid, scalable, and benign (Makarov *et al.*, 2014; Marslin *et al.*, 2018) ^[36, 39].

Among medicinal flora, *Ocimum sanctum* L. (Holy Basil/Tulsi) is an exceptional candidate for green synthesis (Pattanayak *et al.*, 2010) ^[46]. Its extracts feature a dense bioactive profile containing eugenol, ursolic acid, and rosmarinic acid, which possess innate antimicrobial and antioxidant traits (Singhal *et al.*, 2011; Mallikarjun *et al.*, 2011) ^[62, 37]. This rich biochemical repository makes *O. sanctum* a highly efficient matrix for synthesizing stable AgNPs (Philip, 2011) ^[47]. Biochemically, these nanoscale particles easily interact with fungal cell walls, disrupting membrane integrity, inducing reactive oxygen species (ROS), and halting cellular metabolism (Morones *et al.*, 2005; Dakal *et al.*, 2016) ^[41, 18].

This potent antifungal mechanism is desperately needed to combat foot rot disease (quick wilt) in black pepper, caused by the destructive oomycete *Phytophthora capsici* (Anandaraj, 2000) ^[3]. The pathogen decays roots and vines rapidly, causing massive annual economic losses (Truong *et al.*, 2010; Thankamani *et al.*, 2016) ^[67, 65]. Because *P. capsici* is increasingly resistant to conventional fungicides, alternative nanotechnological strategies are vital (Lamour *et al.*, 2012; Elmer and White, 2018) ^[32, 25].

The present study addresses this challenge by synthesizing AgNPs via an eco friendly *O. sanctum* leaf extract pathway, validating their properties using analytical techniques (UV-Vis, FTIR, SEM, XRD, and EDX), and uniformly incorporating them into a composted arecanut shell biofertilizer. By merging green nanotechnology with agricultural waste recycling, this work aims to develop a novel, value added, multi functional agricultural input that simultaneously drives crop nutrition and suppresses *P. capsici*, paving a clearer path toward sustainable plant protection.

2. Materials and Methods

2.1 Chemicals and Other Materials

Analytical grade Silver nitrate (AgNO₃) was purchased from Merck, Darmstadt, Germany. Yeast Extract Potato Dextrose Agar (YEPDA) and Yeast Extract Potato Dextrose Broth (YEPDB) were procured from HiMedia, Mumbai, India.

Infected black pepper plants, soil samples, aging cow dung manure, and fresh and aging areca nut shell samples were collected from Areca Plantations of Bayar village, Kasaragod District, Kerala, India. Fresh and healthy Holy basil plants were collected from Chowki, a village located in the Kasaragod district of Kerala, India.

2.2 Preparation of the Leaf Extract of *Ocimum sanctum* (Holy Basil/Tulsi)

Fresh and healthy Holy basil plants were washed properly three times using distilled water to remove dirt and soil. The plants were then kept for drying in the dark for 15 days. After 15 days, the leaves of the dried plants were powdered using a domestic mixer grinder.

The plant broth was prepared by adding 50 grams of this dried powder into 200 mL of deionized distilled water and boiling the solution for 15 minutes at 60 °C (Philip, 2011) ^[47]. The resulting solution was filtered thoroughly three times using clean filter papers to ensure that no insoluble material appeared in the plant broth. The filtrate was immediately collected in a 250 mL Erlenmeyer flask and stored at 4 °C for further use.

2.3 Phytosynthesis of Silver Nanoparticles from the Leaf Extract of *Ocimum sanctum*

The silver nanoparticles (AgNPs) were phytosynthesized from the leaf extract of *Ocimum sanctum* using adapted protocols (Mallikarjuna *et al.*, 2011; Tailor *et al.*, 2020; Parmar and Singh, 2024; Shinde *et al.*, 2024) ^[38, 63, 45, 59].

Soon after the filtrate was formed, 100 mL of silver nitrate (AgNO₃) solution was immediately added to 150 mL of the filtrate. The resulting mixture was gently shaken for 15 minutes in a shaking incubator and kept undisturbed on the laboratory bench for 2 hours. The resulting sample was subjected to UV-Visible Spectrophotometry and monitored at regular intervals to identify the characteristic absorption properties of silver nanoparticles.

2.4 Preliminary Characterization of the Phytosynthesized Silver Nanoparticles (AgNPs)

UV-Visible spectrophotometry was carried out for the preliminary characterization of the silver nanoparticles (Prema and Kannahi, 2013) ^[49]. To perform this, the sample (mixture of 150 mL filtrate and 100 mL AgNO₃) was diluted with Millipore water 20 times. 150 micro litre of the sample was added to 2850 micro litre of Millipore water to prepare a 3000 micro litre (3 mL) final volume.

Millipore water was used as the blank solution. The absorbance value of the sample at different wavelength ranges (300-320 nm, 320-340 nm, 340-360 nm, 360-380 nm, 380-400 nm, 400-420 nm, 420-440 nm, 440-460 nm, 460-480 nm, 480-500 nm, 500-520 nm, 520-540 nm, and 540-560 nm) was observed using a Shimadzu UV-1780 UV-Vis Spectrophotometer.

2.5 Separation of the Phytosynthesized Silver Nanoparticles from the Sample

After preliminary confirmation of AgNPs formation, the nanoparticles were separated from the matrix using an Eppendorf SE Centrifuge 5430 R (Eppendorf AG, Hamburg, Germany). The sample was centrifuged at 7500 rpm for 20 minutes at 24 °C to separate the *Ocimum sanctum* mediated silver nanoparticles (Anandalakshmi *et al.*, 2016) ^[2].

The AgNPs were washed twice in deionized water at 7500 rpm for 10 minutes at 24 °C. The supernatant was decanted, and the silver nanoparticles settled at the bottom of the falcon tubes were carefully transferred to a clean Petri dish. The nanoparticles were further dried in a hot air oven at 60 °C for 24 hours. The collected powder was stored for Scanning Electron Microscopy (SEM) analysis.

2.6 Physicochemical Characterization

UV-Visible spectrophotometry was used for the preliminary confirmation of synthesis. Scanning Electron Microscopy (SEM) was used to examine the morphology, including the size and shape, of the green synthesized silver nanoparticles to correlate structural features with material properties (Lin *et al.*, 2014) [34]. Dried and purified silver nanoparticles were imaged using a field emission scanning electron microscope (FESEM) (TESCAN MAIA3 XMH, Brno, Czech Republic) at the Sophisticated Analytical Instrument Facility (SAIF), Mahatma Gandhi University, Kottayam, India.

2.7 Collection of Native Antagonistic Fungus from Soil Samples

A fixed plot survey was conducted in Bayar village to identify healthy black pepper plants growing in uninfected areca plantations. Soil samples were collected from the rhizosphere root zones of these plants. Ten grams of each sample was mixed with 90 mL of sterile distilled water and shaken for 5 minutes under aseptic conditions. Serial dilutions (10^{-2} , 10^{-3} and 10^{-4}) were prepared for isolating fungal bioagents (Elad *et al.*, 1980) [24].

One milliliter of each dilution was added to Petri plates containing 20 mL of cooled molten YEPDA medium, mixed gently, and incubated at 28 °C for 7 days. Fungal colonies showing characteristic features of *Trichoderma* spp. were selected, subcultured on agar slants, purified by the hyphal tip culture method, and examined using slide culture techniques to confirm their microscopic characteristics (Rifai, 1969) [54].

2.8 Collection of Plant Pathogenic Fungus from Soil Samples

A fixed plot survey was conducted in Bayar village to assess the incidence of foot rot of black pepper caused by *Phytophthora capsici*. A garden with black pepper vines trained on live areca standards was selected, and plants were assessed for leaf infection, foliar yellowing, defoliation, collar infection, and vine wilting. The pathogen was isolated from infected plant tissues using standard tissue isolation methods after surface sterilization with 1% sodium hypochlorite (NaOCl) (Tsao, 1983) [69]. Samples were cultured on YEPDA medium and incubated at 20-25 °C for 5 days.

P. capsici was also isolated from soil using the traditional castor seed baiting technique (Nambiar and Sarma, 1982) [43]. Surface sterilized castor seeds were buried in infected soil, recovered after 72 hours, resterilized, plated on 2% agar, and the resulting fungal growth was subcultured on slants for further morphological identification.

2.9 Slide Culture of the Fungal Isolates

A sterile slide culture setup was prepared by placing a V-shaped glass rod and a slide inside a Petri dish along with a moistened cotton plug to maintain humidity (Riddell, 1950) [53]. The complete assembly was sterilized at 121 °C for 30

minutes. A 5 mm agar block was aseptically placed on the slide, inoculated with fungal mycelia, covered with a sterile coverslip, and incubated at 28 °C in a BOD incubator. After observing sufficient fungal growth, the coverslip was carefully removed, mounted on a clean glass slide with lactophenol cotton blue stain, and examined under a light microscope for morphological features.

2.10 In vitro Evaluation of Native Antagonistic Fungal Isolates

A dual culture technique was used to assess the antagonistic activity of the *Trichoderma viridae* against the pathogen *Phytophthora capsici* (Morton and Stroube, 1955) [42]. Antagonists and pathogenic fungi were inoculated on opposite sides of YEPDA plates with a 3-4 cm gap and incubated. Pathogen growth inhibition was measured when the control colony reached the edge of the plate (90 mm), and the percent inhibition of radial mycelial growth was calculated using Vincent's formula (Vincent, 1947) [70]:

$$\text{Percent inhibition (I)} = C - T/T \times 100 \quad \text{Eq. (1)}$$

Where I is the percent inhibition of mycelial growth, C is the radial growth (mm) of the fungal colony in the control, and T is the radial growth (mm) in the treatment.

2.11 In vitro Evaluation of Cellulolytic Activities of Fungal Isolates

Raw areca nut shell waste was collected locally, thoroughly washed, dried, and pulverized into a fine powder. A solid state fermentation (SSF) matrix was prepared by amending a basal mineral medium with 1% (w/v) raw areca nut shell powder to serve as the primary lignocellulosic carbon source. The matrix was sterilized by autoclaving at 121 °C for 15 minutes. Upon cooling, the medium was inoculated with a uniform spore suspension of *Trichoderma viride*. Cultivation was carried out under controlled static conditions at 28 ± 2 °C to monitor vegetative colonization and fungal proliferation. The cellulolytic activity and substrate degradation capacity of *T. viride* were evaluated based on the rate and density of vegetative colonization across the dense lignocellulosic skeleton of the areca nut shell matrix. This setup was utilized to assess the suitability of this abundant agricultural waste as a low cost, sustainable organic substrate for mass multiplication and scalable production of the biocontrol agent.

2.12 Preparation of Arecanut Shell Compost Based Biofertilizer

Fresh and partially decomposed arecanut shell samples collected from areca plantations were utilized to assemble the compost based biofertilizer matrix. The shells were washed thoroughly with water to eliminate adhering soil and structural impurities, air dried, and shredded into small pieces to optimize surface area for rapid microbial decomposition. The processed arecanut shells were mixed uniformly with well decomposed cow dung manure, which served as an organic starter source of nutrients and beneficial microbial consortia (Singh *et al.*, 2012) [61].

The mixture was transferred to an outdoor composting pit and maintained under optimum moisture conditions (50-60%) by periodic sprinkling of water. The composting mass was turned regularly to ensure aerobic conditions and uniform decomposition. Composting was maintained until a dark brown, homogeneous material displaying a

characteristic earthy odor was obtained, establishing the structural maturity of the compost (Bhat *et al.*, 2018) [17]. The matured compost was sieved to remove undecomposed macroscopic particles and stored in clean polyethylene bags.

2.13 Incorporation of Silver Nanoparticles into the Arecanut Shell Compost Based Biofertilizer

The silver nanoparticles synthesized using the aqueous leaf extract of *Ocimum sanctum* were integrated into the matured arecanut shell based compost under sterile conditions. Dried AgNPs (1 gram) were weighed and dispersed in 500 mL of sterile distilled water, followed by probe sonication for 30 minutes to generate a stable, uniform colloidal suspension. This nanoparticle suspension was gradually introduced into the compost matrix and mixed thoroughly to guarantee uniform adsorption throughout the organic carrier (Kah *et al.*, 2018) [30]. The AgNPs enriched biofertilizer was allowed to equilibrate for 24 hours at room temperature (27 ± 2 °C) to facilitate thorough interaction of the nanoparticles with the organic substrate matrix before deployment.

2.14 Evaluation of the Antifungal Activity of the Developed Silver Nanoparticles based Biofungicidal Biofertilizer against *Phytophthora capsici* infection in Black pepper

The antifungal efficacy of the AgNPs enriched biofertilizer formulation was evaluated against *Phytophthora capsici* using an agar amendment assay (Lamsal *et al.*, 2011) [33]. A measured quantity (1 gram) of the formulated biofungicidal biofertilizer was incorporated into 500 mL of sterile molten YEPDA medium before plate pouring, while plain YEPDA plates without amendments served as the experimental control.

After solidification of the medium, a 5 mm mycelial disc obtained from the actively growing margin of a 5 day old culture of *P. capsici* was placed at the center of each plate. Inoculated plates were incubated at 28 ± 2 °C, and radial growth of the pathogen was monitored regularly until the control plates achieved full growth. The percentage inhibition of mycelial growth was determined using Vincent's formula [70] (Equation 1):

$$\text{Percent inhibition (I)} = \frac{C-T}{T} \times 100 \quad \text{Eq. (1)}$$

Where I is the percent inhibition of mycelial growth, C is the radial growth (mm) of the fungal colony in the control, and T is the radial growth (mm) in the treatment.

For soil application (around the base) 1 kg of the Developed Silver Nanoparticles based Biofungicidal Biofertilizer was applied per mature pepper vine just before the onset of the monsoon showers (May-June), and repeated the application post monsoon (August-September). The soil must be moist. The soil in the basin was gently loosened (the ring around the base of the vine) and the AgNPs based biofertilizer was applied. Then it was lightly covered with soil or a green leaf mulch to protect the fungus from direct sunlight.

3. Results and discussions

3.1 Green Synthesis of Silver Nanoparticles from the Leaf Extract of *Ocimum sanctum* (Holy Basil/Tulsi)

When 100 mL of Silver nitrate (AgNO_3) was added to 150 mL of leaf filtrate, a rapid visual color transformation from light green to light yellow, then to light brown, and finally to a stable dark brown was observed. This phenomenon is attributed to the surface plasmon resonance (SPR) active behavior that occurs due to the reduction of silver ions (Ag^+) to elemental silver (Ag^0) and the subsequent formation of colloidal silver nanoparticles (AgNPs) accompanied by nucleation and growth stages. This pattern of shifting coloration is a well established early macro indicator of plant mediated nanoparticle synthesis, validating the successful initiation of the green reduction route (Brahmachari *et al.*, 2014) [9].

3.2 Physicochemical Characterization

Preliminary confirmation of the biosynthesis of AgNPs was established via UV-Visible spectrophotometry. The absorption spectrum displayed a distinct Surface Plasmon Resonance (SPR) peak centered around 430 nm (Table 1 and Fig 1). This is highly consistent with the characteristic SPR bands typically reported for stable plant mediated silver nanoparticles. Parallel studies focusing on *Ocimum sanctum* leaf extracts have consistently documented SPR absorption maximums falling precisely within the 420-430 nm range, confirming the uniform generation of nanoscale metallic silver (Deepan *et al.*, 2023) [19].

Table 1: The absorbance value of the green synthesized Silver nanoparticles at different wavelength ranges

Wavelength range (nm)	Absorbance value of Silver nanoparticles (a.u)
300-320 nm	0.31
320-340 nm	0.26
340-360 nm	0.14
360-380 nm	0.28
380-400 nm	0.37
400-420 nm	0.48
420-440 nm	0.59
440-460 nm	0.46
460-480 nm	0.35
480-500 nm	0.23
500-520 nm	0.21
520-540 nm	0.16
540-560 nm	0.11

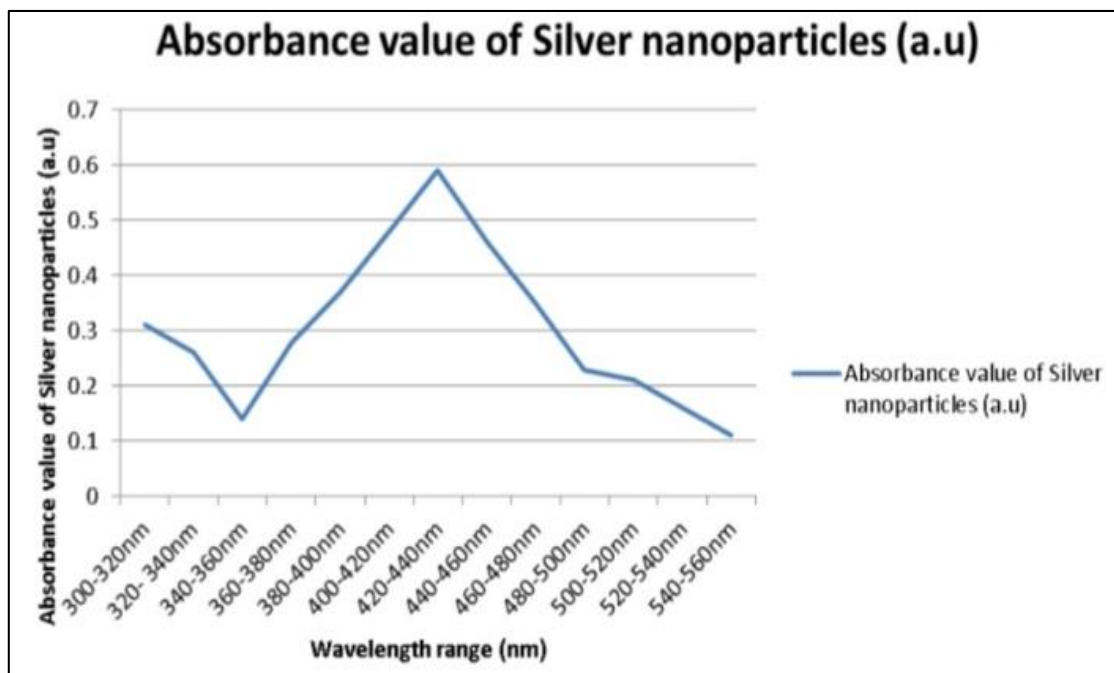


Fig 1: Graphical representation of the absorbance value of Silver nanoparticles at different wavelength ranges.

Scanning Electron Microscopy (SEM) analysis revealed that the synthesized silver nanoparticles possessed a predominantly spherical morphology as seen in Fig 2. The average particle core diameter was measured to be approximately 42 nm. This configuration aligns seamlessly with existing nanotechnology literature, which establishes that green synthesized AgNPs typically range between 10-50 nm depending on the specific organic functional groups and kinetic conditions of the plant extract used (Shahzadi *et al.*, 2025) ^[57].

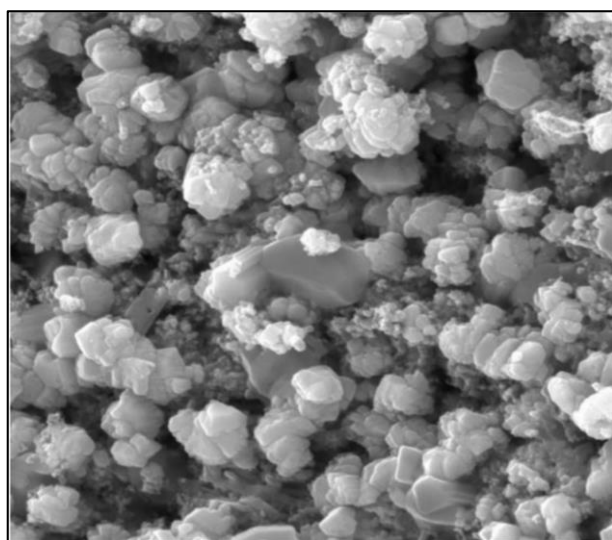


Fig 2: Scanning Electron Microscopy image of the green synthesized Silver nanoparticles.

3.3 Characteristic Features of Native Antagonistic Fungus from Soil Samples

The phenotypic macromorphology and microscopic architectures of the isolated biocontrol fungus *Trichoderma viridae* are detailed across Fig 3 and 4. The fungal cultures exhibited a typical radial, slow initial vegetative growth pattern, achieving a colony diameter of 30 mm within 40 hours of incubation. It developed a characteristic light green

sporulation zone after 48 hours of incubation at 28 °C. The cultures emitted a distinctive, sweet coconut like odor without secreting any visible diffusing pigments into the surrounding agar medium, which is a classic diagnostic feature for this specific species (Samuels *et al.*, 2006) ^[55].

Microscopic examination confirmed dark green conidial structures, a velvety colony texture, and globose, distinctly warted conidia. Differentiated chlamydospores formed structural walls within one week of incubation. The structural setup revealed compact green conidial masses, branched, ball like conidiophore arrangements, and floccose to arachnoid mycelial network growth. Native antagonistic fungi of this nature function as excellent natural biocontrol agents and eco friendly alternatives to chemical synthetic pesticides. The isolated native strain demonstrated high rhizosphere adaptability and structural stability, confirming its prospective value as a bio input for integrated plant disease management regimes (Dennis and Webster, 1971) ^[21].



Fig 3: Colony characteristics of *Trichoderma viridae*

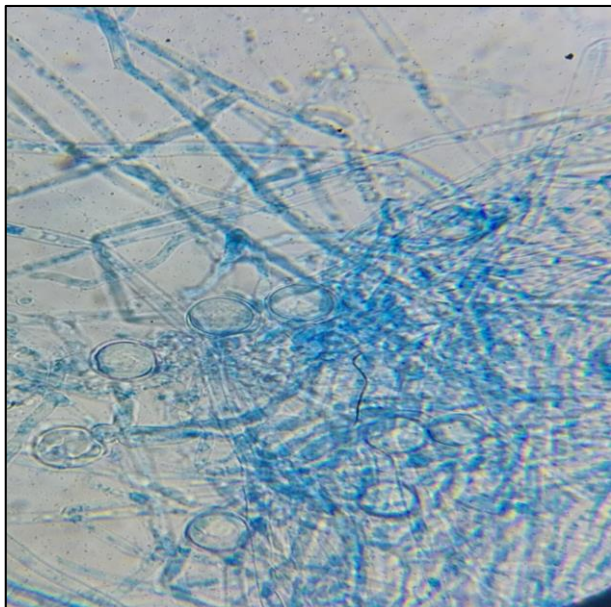


Fig 4: Microscopic structure of *Trichoderma viridae*.

3.4 Characteristic Features of Plant Pathogenic Fungus from Soil Samples

The aggressive pathogen *Phytophthora capsici* was successfully isolated from symptomatic water soaked leaves, necrotic stems, rotted roots, and corresponding rhizosphere soil systems collected across the affected pepper plantations of Bayar village. Following culture on Yeast Extract Potato Dextrose Agar (YEPDA) media, the isolate was purified via precise hyphal tip isolation techniques over a 2% water agar base (Tsao and Menyonga, 1966)^[68].

The morphological characteristics of the isolated oomycete are illustrated in Fig 5 and 6. *Phytophthora capsici* displayed classic oomycete characteristics, showing coenocytic (aseptate) hyphae, highly distinct papillate, lemon shaped sporangia, and characteristically long, deciduous pedicels (Aragaki and Uchida, 2001)^[4]. On YEPDA plates, the isolates demonstrated vigorous vegetative expansion, reaching up to 8 cm within 5 days, exhibiting a dense, white cotton like aerial mycelial pattern.

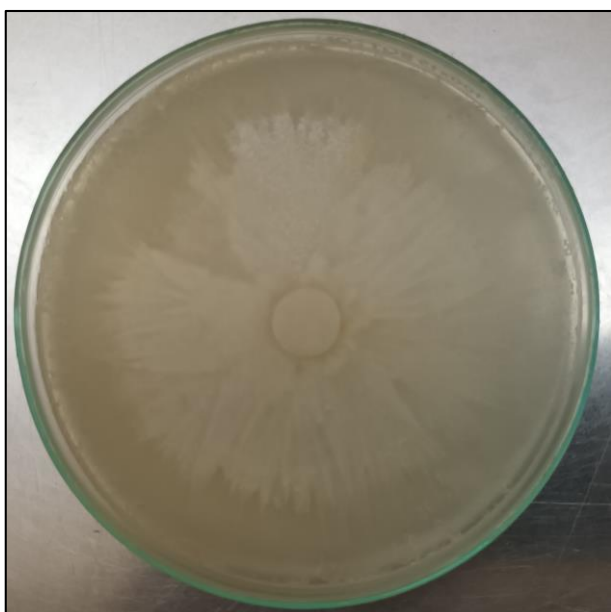


Fig 5: Colony characteristics of *Phytophthora capsici*.

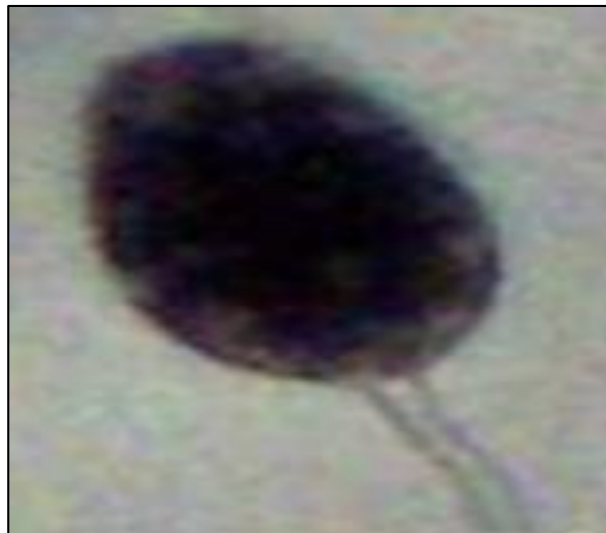


Fig 6: Microscopic structure of Sporangia of *Phytophthora capsici*.

3.5 In vitro Evaluation of Pathogenicity of Fungal Isolates

The specific *in vitro* inhibitory power of the healthy areca plantation derived *Trichoderma viridae* strain against the virulent *Phytophthora capsici* isolate was mapped under strict dual culture conditions (Fig 7). *T. viride* restricted the destructive mycelial advancement of *P. capsici* by 97% relative to control plates.

P. capsici selective isolation from infected tissues was maintained using a specialized PVPH (Pimaricin, Vancomycin, Pentachloronitrobenzene and Hymexazol) amended YEPDA medium to suppress non target zygomycetes and true fungi, while soil borne tracking was executed using the traditional castor seed baiting method (Tsao and Menyonga, 1966)^[68]. The native *Trichoderma* isolates demonstrated superior inhibitory mechanics over typical bacterial alternatives. This enhanced bio efficacy is likely driven by the fast fungal mycelial growth rates, direct hyperparasitic coil formation around oomycete structures, and the volatile release of anti oomycete organic metabolites such as acetaldehyde fractions, which systematically disrupt the cellular integrity of *P. capsici* (Dennis and Webster, 1971)^[21].

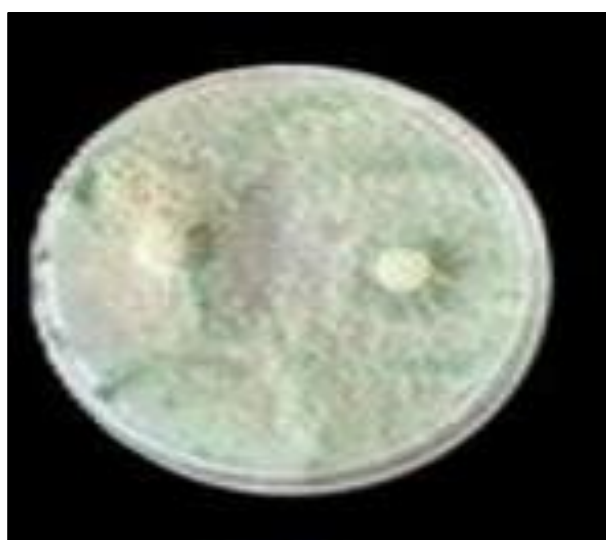


Fig 7: *In vitro* evaluation of *Trichoderma viridae* against *Phytophthora capsici*

3.6 *In vitro* Evaluation of Cellulolytic Activities of Fungal Isolates

T. viride demonstrated highly rapid vegetative colonization on a matrix enriched with 1% raw areca nut shell powder, proving its capacity to degrade tough, complex natural lignocellulosic substrates as seen in Fig 8. Consequently, this abundant agricultural areca nut shell waste was selected as an ideal, low cost organic substrate for the mass multiplication and solid state fermentation of this biocontrol agent. Its local availability and dense carbon skeleton chemistry provide excellent long term nutritive support for fungal proliferation, facilitating its scaled production for economical integrated disease management programs.



Fig 8: *in vitro* utilization of areca nut powder by *Trichoderma viridae*

3.7 Preparation of Arecanut Shell Compost Based Biofertilizer

An organic, nutrient dense compost based biofertilizer was successfully engineered by processing aged arecanut shell fibers blended with well decomposed dairy cow dung. Over the course of the solid state composting cycle, the highly lignocellulosic arecanut shells underwent systematic structural breakdown. This yielded a rich dark brown, loose, highly porous, and structurally homogeneous finished organic compost that released a pleasant, characteristic earthy odor signaling true compost maturity.

The starter inclusion of fresh cow dung significantly accelerated the breakdown kinetics by infusing the pile with a dense consortium of hydrolytic bacteria and essential nitrogen compounds required to optimize the carbon to nitrogen (C:N) ratio. The fully matured compost matrix was completely devoid of ammonia odors or undegraded shell fragments. This renders it an ideal, physically stable carrier vehicle for beneficial biocontrol microorganisms. The successful bioconversion of this rigid agricultural residue into active organic humus highlights its value as a renewable resource rather than a disposal liability. This technique supports closed loop agricultural waste recycling and enhances soil structure when introduced into depleted farming systems. The strong baseline cellulolytic profile of our native *T. viride* isolates underpins this accelerated biodegradation of hard lignocellulosic fractions within the arecanut waste.

3.8 Incorporation of Silver Nanoparticles into the Biofertilizer

The green synthesized silver nanoparticles derived from the aqueous leaf extract of *Ocimum sanctum* were successfully blended into the cured arecanut shell compost matrix to form an advanced, multifunctional nano biofungicidal biofertilizer formulation as seen in Fig 9. Homogeneous blending of the aqueous nano colloid with the solid organic carrier was achieved without displaying any localized particulate clumping, precipitation, or structural phase separation. This implies an exceptionally uniform physical distribution of the active AgNPs throughout the complex organic porous matrix.

The logical integration of metallic silver nanoparticles is designed to provide a synergistic performance spike, imbuing the organic fertilizer with an active, long lasting defense shield against soil borne phytopathogens without degrading the core nutritional value of the compost. Plant mediated AgNPs are highly valued for their broad antimicrobial activity, owing to their elevated surface area to volume ratios which allow for superior contact options against invading microbial cells. Furthermore, the residual protective coating of *Tulsi* phytochemicals remaining on the nanoparticle shell acts as a structural buffer, preventing oxidation or structural breakdown within the biochemically active organic compost matrix (Shahzadi *et al.*, 2025) [57].

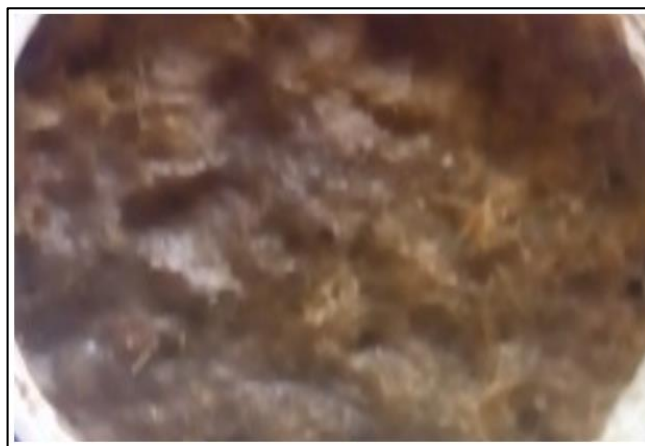


Fig 9: Developed AgNPs based Biofungicidal Biofertilizer

3.9 Evaluation of the Antifungal Activity of the Developed AgNPs based Biofungicidal Biofertilizer

The final protective bioefficacy of the synthesized AgNPs fortified biofertilizer was tested against *Phytophthora capsici*, the highly destructive causal agent behind foot rot epidemics in black pepper. The pathogen *Phytophthora capsici* has shown 100% inhibition as seen in Fig 10. The finished composite formulation achieved profound, highly significant inhibition of the oomycete pathogen under *in vitro* challenge trials, confirming its potential as a targeted biofungicide. This protective response is driven by the combined, multi target action of the biogenic silver nanoparticles working in tandem with the biologically active metabolites of the cured compost. Together, they create an inhospitable micro environment for oomycete growth and zoospore germination.

Silver nanoparticles exert their target fungicidal effect by anchoring to and penetrating the fungal or oomycete cell wall and plasma membrane structures. This induces severe physical deformity, alters membrane permeability

coefficients, and deactivates crucial respiratory enzymes. Once inside the cytoplasm, AgNPs trigger systemic intracellular cascades that generate overwhelming amounts of Reactive Oxygen Species (ROS), inducing critical oxidative stress, lipid peroxidation, and fatal DNA damage within the pathogen cells (Shahzadi *et al.*, 2025)^[57].

When translated to field conditions as a split dose soil application (1 kg/vine) timed with the pre and post monsoon showers, the formulation established excellent protective dynamics in the black pepper rhizosphere (root zone). Loosening the basin soil and applying a protective leaf mulch created an ideal, high moisture microenvironment that shielded the living component of the biofertilizer from UV degradation. Consequently, treated mature pepper vines exhibited high resilience against foot rot (*Phytophthora* wilt) despite heavy monsoon pressures as seen in Fig 11. Vines under this application regime maintained robust

canopy architecture, displayed negligible leaf yellowing or defoliation, and showed active new root flushes. By outcompeting soil pathogens and strengthening the root zone, the dual action formulation effectively functioned as both a reliable bio shield and a growth stimulant under natural field conditions.

Concurrently, the organic compost component acts as a slow release nutrient reservoir that stimulates indigenous beneficial rhizosphere microorganisms while optimizing the soil's physical parameters, thereby promoting robust root vitality and systemic acquired resistance (SAR) within the host plant. Consequently, merging green nanotechnology with recycled arecanut shell media offers an innovative, dual action path toward sustainable crop protection. It simultaneously meets plant nutritional requirements and manages devastating soil diseases, helping to phase out toxic synthetic chemical inputs.

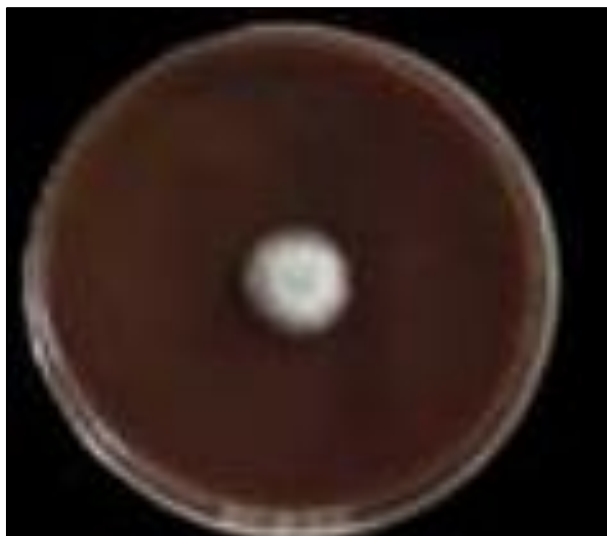


Fig 10: Plate showing complete (100%) inhibition of *Phytophthora capsici* by the developed AgNPs based Biofungicidal Biofertilizer



Fig 11: Healthy and disease free Black pepper plants grown after the application of the developed AgNPs based Biofungicidal Biofertilizer.

4. Conclusion

The present study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Ocimum sanctum*, providing an eco-friendly

and sustainable alternative to conventional nanoparticle synthesis methods. The formation of AgNPs was confirmed by a characteristic colour change, UV-Visible spectroscopy, SEM, and EDX analyses, which indicated the successful production of predominantly spherical nanoparticles with an average size of 42 nm. An organic biofertilizer was successfully prepared from arecanut shell waste and incorporated with the synthesized AgNPs to develop a value-added biofungicidal formulation.

Native *Trichoderma viride* isolated from healthy rhizosphere soil exhibited strong antagonistic and cellulolytic activities, supporting its potential role in biological control and biodegradation of lignocellulosic arecanut shell waste. The developed AgNP-enriched biofertilizer showed promising antifungal activity against *Phytophthora capsici*, suggesting its potential application in the management of foot rot disease in black pepper. Overall, the study highlights the possibility of integrating plant-mediated nanotechnology with agricultural waste recycling to develop an environmentally friendly agricultural input that supports both crop nutrition and disease management. This approach offers a sustainable alternative to chemical fertilizers and fungicides while promoting the effective utilization of arecanut shell waste and contributing to environmentally responsible agricultural practices.

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