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Rapid Synthesis of Silver Nano Particles Capped In Starch and its Anti - Mold Activity

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ABSTRACT: A rapid preparation of nanomaterial of noble metal (Silver) via chemical reduction is presented. Silver nitrate (oxidizing), glucose (reducing) and corn starch was the capping agents.
Corn starch stabilized Silver Nanoparticles (AgNPs) synthesized rapidly in aqueous system via the assistance of microwave irradiation had its Surface Plasmon Resonance (SPR) monitored by a UV-Visible Spectrophotometer and peaked at 400nm. Further characterization was by FT-IR and TEM/ SAED. Self-assembly of starch-capped silver nanoparticles gave a mirror-like glassy film surface on drying. According to the FT-IR spectroscopy, AgNPs revealed the shifting of N-H and O-H of starch still exists. TEM gave average particle size of 7nm. Result also showed that AgNPs can inhibit the growth of mold (A. *niger*). Method is reliable, ecofriendly, cheap and evident by the properties of the nanoparticles obtained.

KEYWORDS: Starch, Silver nanoparticles, Microwave, SPR and anti-mold.

1 INTRODUCTION

The many properties of silver is well known and the synthesis of its nanoparticles (usually ranging in size from 1 to 100 nm), has become prevalent because its nanoparticles is being used for numerous applications, physically, medically/ pharmaceutically and biologically. They have been reported to have a larger surface area than macro-sized materials as well as presenting new properties which has earned them the several applications. A number of synthetic methods have been employed for the synthesis of silver-based nanoparticles involving physical, chemical and biochemical techniques [1]. Silver Nanoparticles (AgNps) has found application in the purification and quality management of air, biosensing, imaging, drug and pesticide delivery systems as well as in coatings for solar energy absorption, as optical receptors, as catalysts in chemical reactions and as antimicrobials. With increasing focus on green chemistry, natural compounds like glucose, chitosan, starch, have attracted considerable research interest as safer alternatives, reducing and stabilizing agents to synthesize the silver nanospher [2], [3], [4]. The most popular preparation of silver colloids is chemical reduction of silver salt which may appear simple, but great care must be exercised to make stable and reproducible colloid. Starch has been shown as a good capping agent for many work on inorganic nanoparticles such as gold and silver to form inclusion complexes [1]. Starch is one of the most abundant materials on earth due to its cheapness, it is widely-used in stabilizing and controlling size and shape of metal nanoparticles [5]. The possibility of Preparing nano-sized metals and metal oxides, mainly silver (Ag), titanium dioxide (TiO2), zinc oxide (ZnO) and cooper II oxide (CuO), has brought about the development of novel ranges of biocides.

AgNps biocidal action against microbial has been proposed to be that Ag interacts with the -SH groups of proteins on the cell walls, thereby blocking respiration and causing death [6]. Other reports have that "pits"are formed in the cell wall of bacteria, thereby causing permeability and resulting in death [7]. Yet another explains silver ions form metal-organic complexes and insoluble compounds with the sulphhydryl groups in cell walls of bacteria and fungi, inhibiting metabolism and electron transport by making essential enzymes dysfunctional [8].

Silver is safe and non-toxic to animal cells and highly toxic to bacteria such as *Escherchia coli* (*E. coli*) and *Staphylococcus aureas* as well as fungi [9]. Nano silver in the form of powders as well as suspensions, due to the high surface to volume ratios, has been used as anti-bacterial because it enables the loading of small quantities of silver and thus makes the product cost effective [10],[11]. Molds live everywhere—on logs and on fallen leaves, in the soil/dust and in moist places like bathrooms and kitchens

Spoilage by mold is a serious and costly problem for many industries, be it food processing, wood, pharmaceutical, building, textiles and agricultural. The use of preservatives has become an attractive means to diminish the spoilage due to mold and other microbes. It has been reported that losses due to mold spoilage in the bakery industry averages about 200 million pounds per year in certain countries [12]. The commonest molds found in most tropical environment are: *Rhizopus sp., Aspergillus sp., Penicillium sp., Monilia sp., Mucor sp. and Eurotium sp*. This is due to the climate (seasons), warm and wet. In developing countries, mold growth on products are a serious problem that results in economic losses and made worse by incessant power outage making refrigeration a difficult preservative method. Recently, several means of preservation has been reported for instance, bio-preservation where Lactic acid bacteria (LAB) has been used in the food industry [13]. Also the study on the potentiality of LAB strains to inhibit mold growth and found that only four strains out of 95 tested had anti -fungal activity, while the growth rate of mycelia of several strains of *Penicillium* (*P.chrysogenum, P.corylophilum) and Aspergillus flavus* to temperature and water activity (aW) on sponge cake and observed a dependence on the two parameters [14], [15]. The review [12] of the microbial spoilage of bakery products and their control by preservatives concluded that mold spoilage is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Generally speaking, losses due to mold spoilage have been resulting in lost revenue to the food (packaging, baking and processing) as well as other industries. This work set out to find the possibility to inhibit and control mold growth in typical tropical climate (Nigeria) so as to extend the shelf life of many products of great economic importance. Molds not only cause food spoilage, it has been implicated in some diseases in man and animals mainly due to allergy sensitivity to their spores. Food-handling workers are particularly at risk if they are allergic to mold [16] and [17]. Therefore this study focused on a rapid synthesis of AgNps stabilized by starch and evaluated the anti-mold effect of AgNps on food spoiling mold.

2 EXPERIMENTAL

2.1 MATERIALS

The Corn (CO) seeds were purchased from Dutse market in Abuja.

2.2 REAGENTS

Silver nitrate and glucose were purchased from Finlab Scientific Company Abuja Nigeria. All reagents were of analytical grade and used without further purification. Also solutions were prepared using distilled water. The mold strains were obtained from a 4days mold culture in the laboratory.

2.3 STARCH ISOLATION

A method [18] was adopted with modification (as there was no bleaching) during the starch extraction. The starch was then defatted before use. This is termed "native starch" and had no modification whatsoever.

2.4 SYNTHESIS OF CORN STARCH SILVER NANOPARTICLES (CO-AGNPS)

All materials in our experiments are biocompatible and environmental-friendly. According to a slightly modified methods of [19]&[20], 1ml of 1% solution of $AgNO_3$ was added was mixed for 10mins, with 4ml of 0.5M glucose solution, stirring carried out to make sure both oxidant and reductant were in contact, to this, 1% dispersion of the Corn starch (CO) in distilled water was added and the complex was micro waved(Samsung 123 HCE, TDS) for 5 minutes at 100% power, 800W at 250MHZ working condition. The Oxidant ratio to reductant was 1:4. The setting of the microwave timer for a longer period and then stopping it after 5 minutes enhanced the heating and the solution was quite hot. The complex was allowed to cool and was centrifuged at 11,000rpm for 20minutes using Eppendorf 5417R micro-Centrifuge. The resultant nanoparticle was oven-dried, ground to powder, stored in a closed dark container devoid of sunlight.

2.5 DETERMINATION OF THE FORMATION OF SILVER NANOPARTICLES COLOUR

The colour of the sample after synthesis was checked with naked eyes to examine the formation of Corn starch Silver NanoParticles (COAgNPs).

Possible equation for the reaction during the synthesis:

$Ag_2O + CH_2OH\text{-}[CHOH]_4\text{-}CHO + 2(\text{starch}) \rightarrow CH_2OH\text{-}[CHOH]_4\text{-}COOH + 2Ag(\text{starch})\text{ppt}$

Glucose, as an aldehyde, was able to reduce Ag^+ ions to Ag^0, and through this reaction, glucose can be oxidized to gluconic acid.

2.6 ANTI-MOLD ACTIVITY OF AGNPS

The anti-mold activity of silver nanoparticles was evaluated using the plate spore and spot disappearance method. In duplicate, AgNps was prepared (A) and as well, the control (B) was prepared in the same way but without silver nitrate and glucose solutions just 1% starch dispersion in distilled water was heated in the microwave for 5mins this gave a colloid. Aliquots (50ml) of both A and B colloidal gels were poured into sterile plates and according to the method of [14], conidial suspension of natural food spoiling mold strains *A. niger* (10^4 conidial/ml per 50ml of colloidal gel) was surface –sprayed and plates covered with polythene bags and kept isolated in the laboratory at 30^oC. The plates were observed daily for the manifestation of mold growth so that stability and susceptibility of both AgNps and control gels will be defined as the number of days mold spots appeared on the gels. In another set up, plates A2 and B2 containing 50ml of AgNps and the control were covered with polythene bags at 30^oC and left for days to monitor for their susceptibility to food spoiling mold strains to grow naturally on the starch gels for days. Plates A2 and B2 were also observed daily for the manifestation natural mold growth.

Finally, the susceptible plates were surface –smeared with 5ml of AgNps suspension and monitored for lethal activity of mold strains based on spores and spot disappearance on the plate's surface. This was also monitored for days for possibility of re-manifestation.

2.7 CHARACTERIZATION METHODS

UV-Vis Spectroscopy**:** The prepared colloidal Ag nanoparticles were characterized by 7000 series, CECIL CE 7500 UV-VIS - spectrophotometer. The scanning range was 200-700 nm and the correction of the spectrophotometer was carried out by using distilled water as blank reference. The wavelength corresponding to maximum absorption spectra of the sample was recorded.

Transmission Electron Microscope (TEM) was employed in determining the morphology and size of the particles synthesized.

Fourier Transform Infra-Red (FTIR) Analysis.

The obtained silver nanoparticles (dried samples) were grinded with KBr pellets and used for FTIR measurements. The spectrum was recorded in the range of 4000 - 400 cm-1 using Horizon, Model: MB 3000 spectrometer in the diffuse reflectance mode operating at resolution of 4 cm-1.

3 Results and Discussion

Initially, a colourless clear solution appeared at room temperature even with the rigorous stirring. This then turned yellowish brown on heating for 5minutes in the microwave. The change in colour may be due to excitation of surface Plasmon vibrations of silver nanoparticles. The step-wise formation of the AgNps is shown in figure 1. These colour changes indicates the formation of colloidal Silver (Ag) nanoparticle. Interestingly, microwave heating was able to initiate a reduction of Ag^{+} to Ag^{0} when glucose was employed as a reductant. Meaning that as temperature increases, the Surface Plasmon Resonance (SPR) typical of silver nanoparticles appears [19].

3.1 UV-Vis Analysis (Kinetics of Formation of AgNps)

UV-VIS absorption spectra have proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibit an intense absorption peak due to the surface plasmon (it describes the collective excitation of conduction electrons in a metal) excitation [21]. The Uv-vis absorption spectra of corn starch AgNps is presented in Fig.2. Formation of strong absorption band centered at 400nm clearly suggests formation of Ag nanoparticles embedded in the starch matrix. For the broadening observed, according to literature broad peaks in the beginning of formation of AgNps, is attributed to very small particles (seeds) [21]. This is in agreement with [6], that employing microwave assistance in synthesis yields relatively small nanoparticles in a short reaction time. This observation is further confirmed by TEM analysis. A microwave synthesis of silver nanoparticles involves the reduction of silver nanoparticles using variable frequency microwave radiation as against the conventional heating method. The method yields a faster reaction and gives a higher concentration of silver nanoparticles with the same temperature and exposure [20].

Fig. 1: Stepwise preparation of CO-AgNPs. The Color changes of reaction complexes containing colloidal AgNPs in a microwave assisted method (A) at the beginning, (B) during microwave irradiation, (C) and the resultant complex after 5minutes of microwave heating.

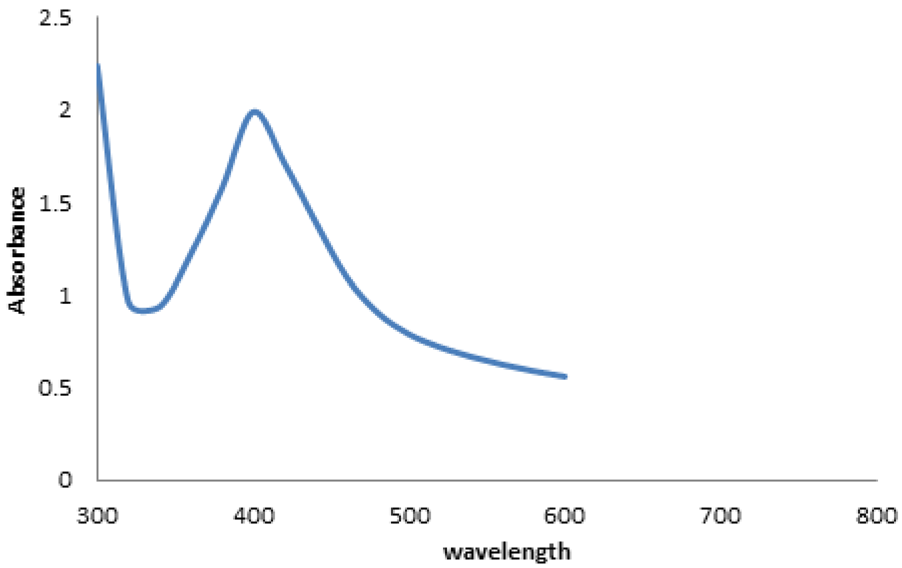

Fig. 2 showing the UV-Vis spectra of the starch-AgNPs

3.2 MORPHOLOGIES

The Transmission Electron Microscopy (TEM) images of silver nanoparticles embedded in the corn starch Ag/CO/NPs is presented in Figure 3. The image reveals spherical particles of average size range 7-15nm, majority of the spheres are in the size range of 7-15nm with a few above 25-30nm.. With the average particle size obtained from these micrographs being about7.6nm ± it is obvious that these nanoparticles are polydisperse, aggregated, and the Selected-Area Electron Diffraction (SAED) patterns depicted in Figures 3b shows bright dots, indicating that these nanoparticles may have crystalline in nature. Usually, the arranged rings can be attributed to the diffraction from the (111), (200), (220), and (311) planes of Face-Centered Cubic (FCC) silver [22]. TEM micrographs are the best method of determining morphology of nanoparticles and the obtained spheres are in agreement with the report of [19], [6], that microwave assisted synthesis yield small particle size nanoparticles.

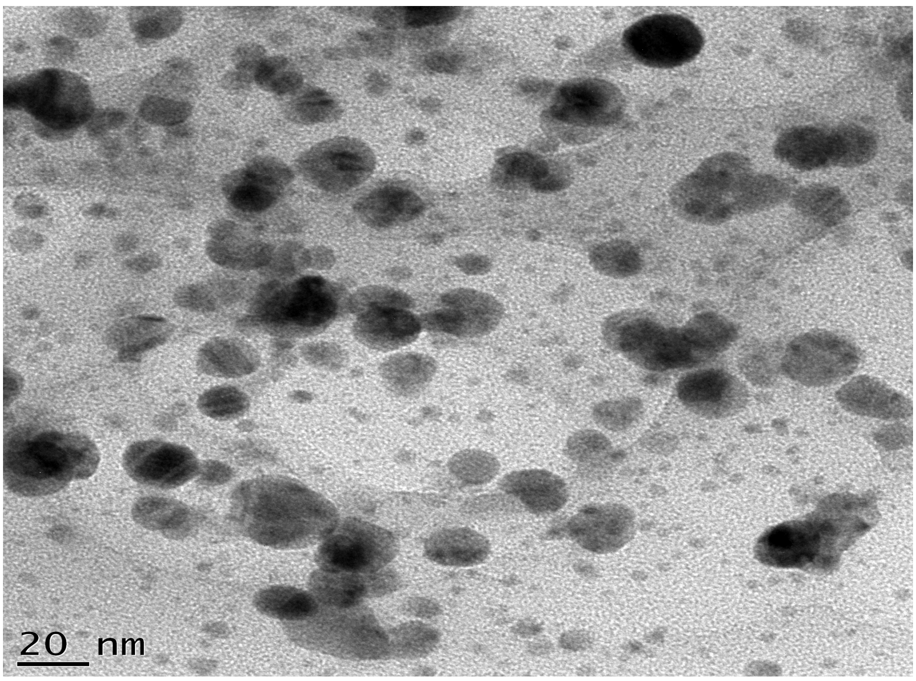

Figure 3 Showing Transmission electron micrograph images of AgNPs synthesized.

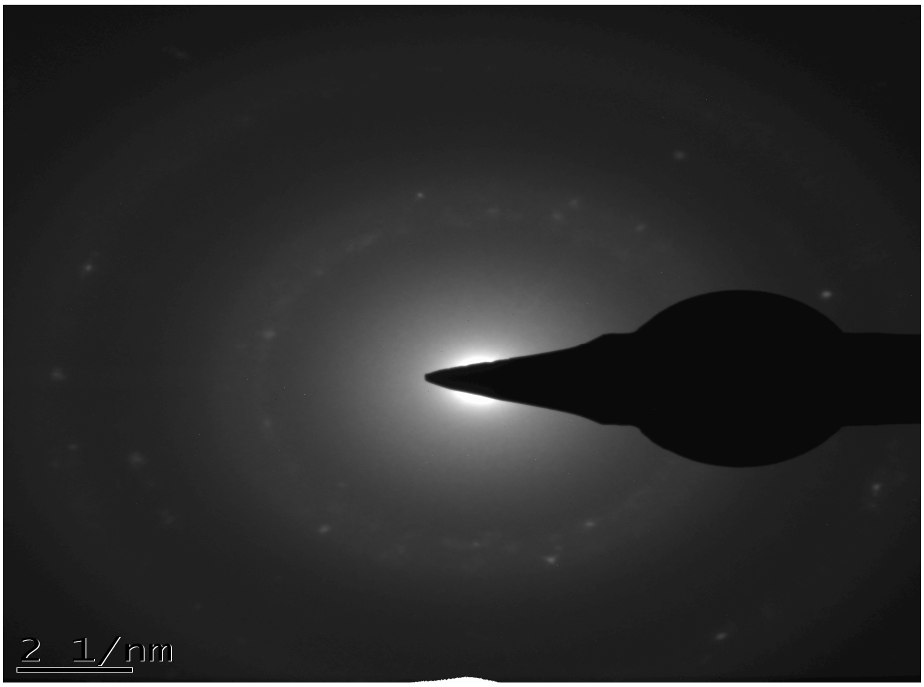

Figure 3b showing the bright dots of the silver nanoparticles in the SAED.

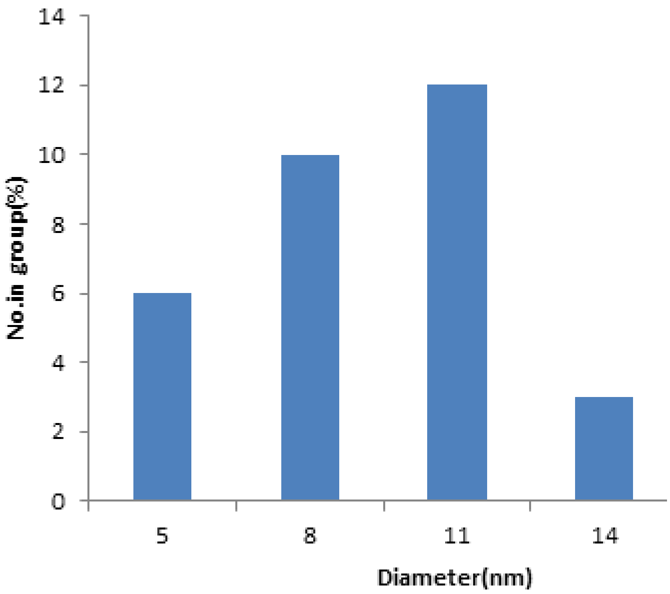

Fig.3c Particle size distribution plot for silver nanoparticles prepared by microwave heating.

3.3 FOURIER TRANSFORM INFRARED (FTIR) CHARACTERIZATION

The FTIR spectra of the starch silver nanoparticles were recorded in order to identify the functional groups of glucose and starch involved in the reduction and capping/stabilization of the synthesized nanoparticles. Figure 4 shows the Fourier transform infrared spectrum for the synthesized Silver nanoparticle revealing vibrations of ether and alcoholic groups as major absorbance bands respectively. It has been reported [23] that 3600–2800-cm^{-1} region, strong hydrogen-bonded (O–H) stretching absorptions and weak C–H stretching absorptions are usually observed. That played out as our spectrum had characteristic broad and strong absorbance bands at 3404.47cm^{-1}, 1643cm^{-1} and 2926 cm^{-1} which could be assigned to the hydroxyl (O-H) group and C-H stretching vibrations respectively. A shift from 3385 cm^{-1} to 3404 cm^{-1} is observed for stabilized AgNPs; this may be due to the inter and intra molecular interactions of Ag^0 with –OH group. There are peaks at 1155 cm^{-1} , 1230 cm^{-1} , 1022 cm^{-1} , and 875 cm-1. They maybe gluconic acid peaks [24].

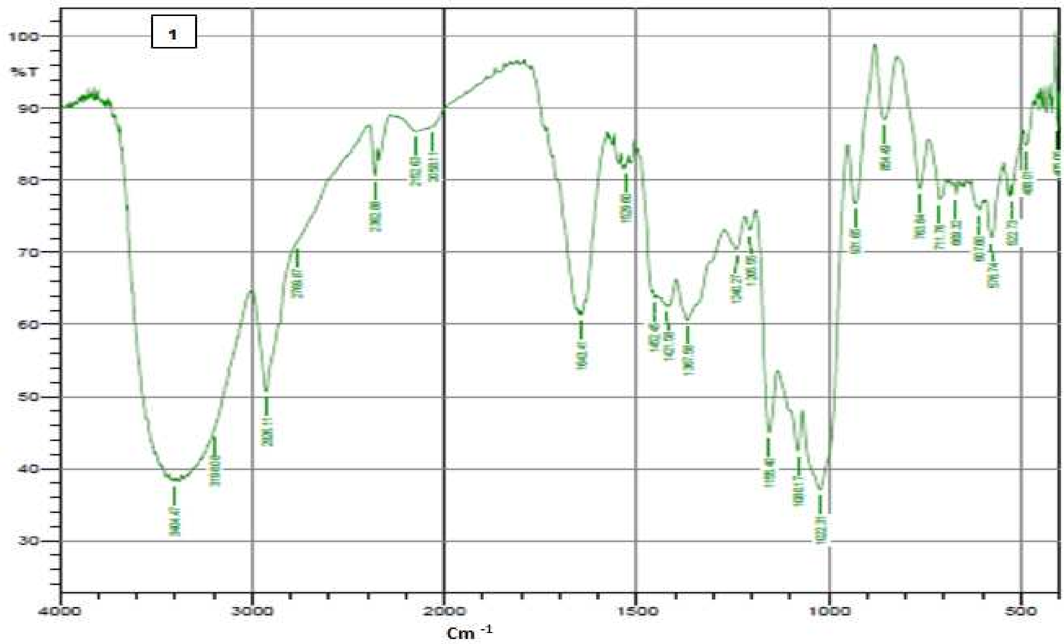

Fig. 4(1) showing the FTIR spectral of silver nanoparticle synthesized via microwave heating.

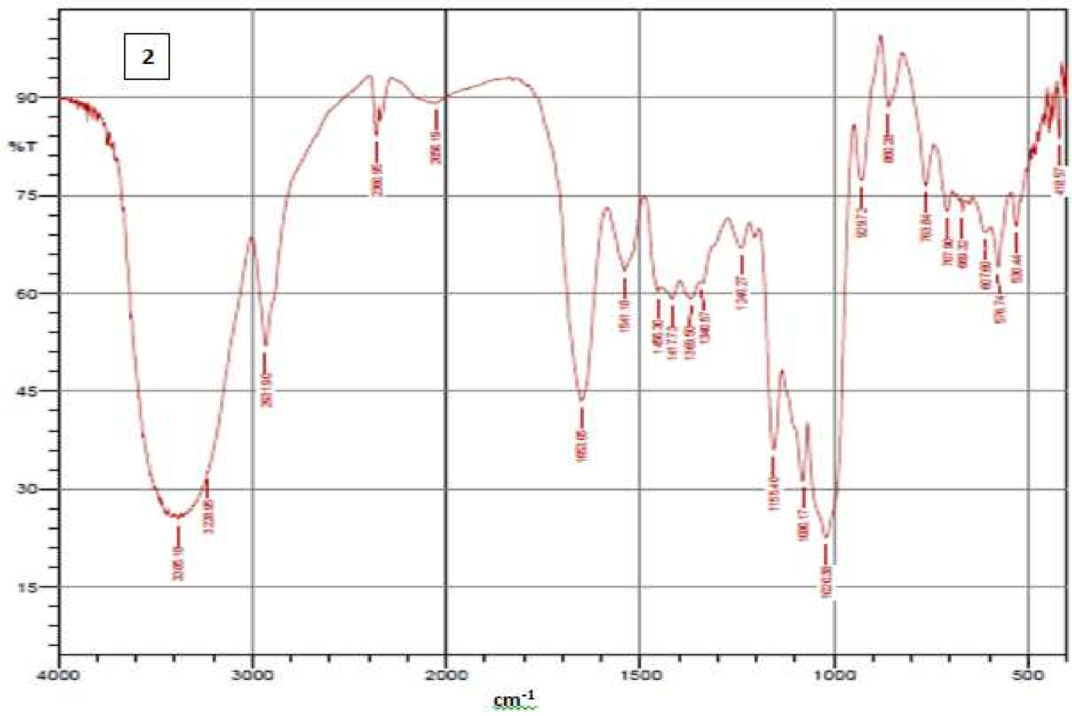

Fig. 4(2) showing the FTIR spectral of native starch

3.4 ANTI- MOLD ACTIVITY

Figure 7; shows plates to which AgNPs and the control colloidal suspensions (50ml each) was applied (plates A&B). In the plates with AgNPs, presence of silver nanoparticles inhibited modal growth by 100%. As the control was found to be susceptible to different strains of the *A. niger* which was visible in coloured spots (blue, grey, whitish) on the plate (C). The measure of inhibition zones is the entire circumference of the plates (90mm). Anti mold activity was also assessed by smearing the surface of the mold infested plates with AgNPs colloids (5ml) which revealed a 24hour lethal action as mold spots were found to be disappearing daily and no new manifestation observed for months. The grown strains of A*. niger* in plate C, were eliminated and further growth inhibited in the plate (D). The test was repeated at least twice, for each treated sample. AgNPs samples presented good Anti- mold activity. Plate E depicts the end product AgNPs films as obtained on drying.

MODE OF ACTION

Though there are many mechanisms attributed to the antimicrobial activity shown by silver nanoparticles, the actual and most reliable mechanism is not fully understood or cannot be generalized as the nanoparticles are found to act on different organisms in different ways. Silver has always been an excellent antimicrobial and has been used for the purpose for ages. The unique physical and chemical properties of silver nanoparticles only increase the efficacy of silver [25]. The mode of antimicrobial action of silver ions is presently limited to the effect on their structure and morphology. A study summarized it to be linked to the DNA state at a time. During relaxation, replication occurs while replication is lost when DNA is condensed. Hence, when the silver ions penetrate inside the microbial cell the DNA molecule turns into condensed form and loses its replication ability leading to cell death [10].

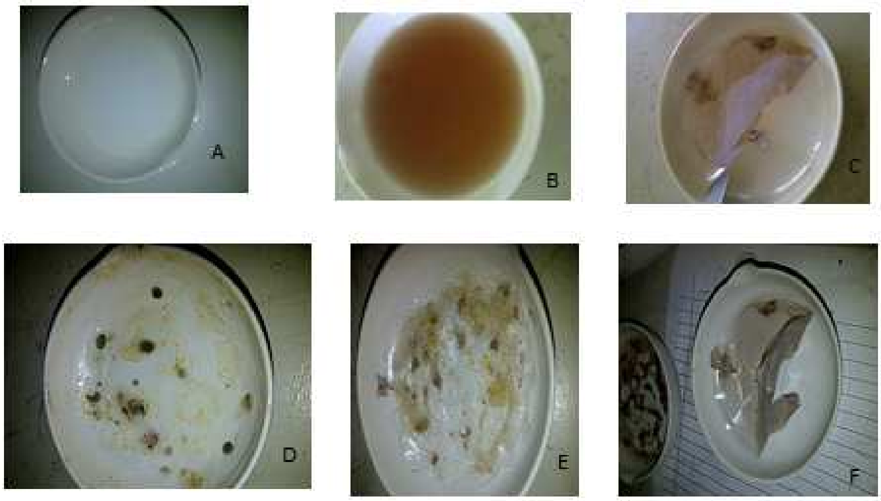

Figure 7. (a-f) Representative photographs of fungicidal activity against bread mold (A. niger), where: a and b are the resultant colloids, c is the dired product of B (AgNPs). While D is the mold infested plate, E the AgNPs smeared plate undergoing inhibitory Mode fungal growth after 24 hours and F the dried plate d after all mold appearances has been eliminated.

Figure 8: A comparison of the untreated and treated surfaces of mold infested plates. Plate 2 is undergoing lethal and inhibitory action by the smeared AgNPs.

4 CONCLUSIONS

A critical need in the field of nanotechnology is the development of reliable, fast and eco-friendly processes for synthesis of metallic nanoparticles. Here, we have report a simple rapid, chemical and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with glucose under microwave irradiation. Starch was employed as a stabilizing agent. The characteristics of the obtained silver nanoparticles were studied using UV-Vis, FTIR, and TEM techniques. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. The silver nanoparticles had mean diameters of 7nm.

Additionally, results reveal that AgNPs synthesized has considerable anti-mold activity, a fact that can be related to size of colloidal silver particles. Also, the antimicrobial susceptibility of silver nanoparticles synthesized was investigated. The smeared spore and spot disappearance method was used as antimicrobial inhibitory and susceptibility testing method. Areas of inhibition were measured after 24 hours of surface –smearing. The comparison of treated and untreated plates showed an almost magical clearance of the mold on plate 2 in figure 8. We have been able to show in this present study, obtained results of antimold activity reveal that the growth of A. *niger* was inhibited at colloidal volume of 5ml/90mm plate size.

This study shows that, Ag-NPs synthesize from a cheap, non-toxic, and renewable and generally compatible natural polymer (starch) could considerably be used in industries without placing pressure economically.

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