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Evaluation of the effect of green synthesized silver nanoparticles on dyeing process and *in vitro* contamination control of Egyptian cotton

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Abstract

Biosynthesis of silver nanoparticles (AgNPs) using bark extract of *Acacia nilotica* as green reducing agent and natural colorant was achieved. Simultaneous dyeing of Egyptian cotton fabric and control of the in vitro contamination of Egyptian cotton culture (var Giza 86) plant were also carried out. Ferric chloride was used as metal mordant in the dyeing process and the color measurements were carried out to evaluate the shade obtained. The dyed fabric was subjected for analysis in terms of K/S, CIE L*a*b*, and fastness properties. For assaying the antimicrobial activity, the disk diffusion assay on two model organisms, *E. coli* (Gram Negative) and *Bacillus subtilis* (Gram-positive) were achieved resulting that the treated cotton fabric showed a clear antimicrobial against both model organisms. The treatments of produced AgNPs on cotton not only improve its antimicrobial efficiency but also influenced the tensile strength of the fabric sample positively. The treatment was found to enhance the color depth and fastness properties of dyed cotton fabric samples. Concerning the new application for in vitro control of bacterial and fungal contamination, the synthesized AgNPs was conducted in the sterilization protocol and showed the effective elimination of fungal and bacterial contamination in in vitro cotton var G86, these results provided a new sterilization product to be used for future studies of plant tissue culture.

Keywords: Acacia; AgNPs; cotton fabric; *In vitro* Contamination; antimicrobial properties

1. Introduction

Today, the nano science can easily be presumed as the key feature of modern world technology. Therefore, due to assorted field of utilizations, it is playing pivotal role in material science industry [1]. Nanotechnology is referred to the term for manufacture, portrayal, manipulation and application of structures by controlling shape and size at nano scale [2]. The field of nanotechnology is the most dynamic region of research in material sciences and synthesis of nanoparticles (NPs) is picking up significantly throughout the world.

Metal Nanoparticles are the most dominate between inorganic nanoparticles and defined as particles with a diameter between 1 and 100 nm. Smaller nanoparticles less than 10 nm in diameter are creating a new category of materials whose properties are quite unique and different from the corresponding bulk or from atoms [3]. Many methods are applied for synthesis of metals nano forms such physical, chemical and biological actions. The produced nanomaterials forms, size and action may change from method to other depend on many parameters as capping agents, reducing system, stabilizing materials and environmental conditions [4].

Nanoparticles have a lot of applications in environmental science including environmental remediation, hazardous waste management, metal biosensors, antimicrobial, and removal of heavy metals and decolorization of dyes. Because of nanomaterials have high surface/volume ratio, it has been explored to detect and treat pollutants in various environmental matrixes like wastewater, soil and sediment by means of adsorption, oxidation–reduction, surface complication and other mechanisms [5,6].

Silver nanoparticles are domineering among the most important and entrancing nanomaterials among few metallic nanoparticles that are engaged with the biomedical applications. They exhibit

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excellent antibacterial, antifungal, anti-inflammatory and antiviral properties generously or either after reacting with specific elements to impart such functional properties [7,8].

Apart from conventional Ag^{+}, silver is available in three other oxidation states: Ag^{0}, Ag^{2+}, Ag^{3+}. However, the last two are unstable and rarely found in the sea-going condition. Silver has many isotopes but with molecular weight 107. Even though intense poisonous quality of silver in the condition is subject to the accessibility of free silver particles [9].

Recently, researchers have shown that the silver nanoparticles interact with a human immunodeficiency virus and prevent virus from binding to the host cells [10]. The antimicrobial activity of silver nanoparticles is comparatively better than the broad spectrum most prominent antibiotics used worldwide. Fiber surface charge depends upon their chemical and physical-chemical structure, swelling capacity as well as upon iongenity, structure and concentration of adsorbent. Different treatments, mostly alkaline modifications of fibers, change surface charge and adsorption ability. Adsorption properties of fibers are influenced by change of surface charge [11].

Nano-silver, when in contact with microorganisms and their growth, unfavorably influences the cell digestion of the electron exchange structures and causes the substrate movement in the microbial cell film [12,13]. Microscopic organisms and growth causes irritation, contamination, smell, wounds, the utilization of nano-silver subdue the expansion of microscopic organisms and parasites [14]. Both, the Gram-positive and Gram-negative microorganisms are successfully killed by nanosilver, so it can call as executing agent including the anti-toxin safe strains [15]. Gram-negative microscopic organisms are the microbes hold the shade of the stain even the wake of washing with liquor acetone or alcohol as well include genera, for example, *Salmonella*, *Acinetobacter*, *Escherichia*, *Vibrio* and *Pseudomonas*. The little-sized nanoparticles with a vast surface range to volume proportion give a more effective intends to antibacterial action even at low fixation. Additionally, the antimicrobial action of silver nanoparticles relies on the fixation and shape. Because of their expansive surface zone to volume proportions, truncated triangular silver nano-plates show the most grounded antibacterial action [16].

Recently, many studies reported that, treated cotton fabrics with nanocrystalline silver particles using pad-dry-cure method. More recent researches were interested in manufacturing of multifunctional fabrics by uploading of AgNPs through exploiting fabric backbones with its reducing end groups to play the dual roles of reducer and capping agents for metal nanoparticles [17]. In addition, using the non-toxic and eco-friendly natural dyes on textiles has a significant importance because of increasing the environmental awareness with a view to avoid the hazardous synthetic dyes. However, the use of natural dyes for the coloration of textiles has mainly been limited to craftsman, printers, small scale dyers, small scale exporters and producers treating with high valued eco-friendly textile production and sales [18]. Natural dyes are known for their use in colouring of food substrate, leather, wood as well as natural fibers like wool, silk, cotton and flax as major areas of application since ancient times [19].

Silver nanoparticles have special properties leading them to be one of the most efficient and commercialized nano materials for health care. The natural antibacterial agents which acting against aquatic and nonaquatic microorganisms makes it attractive in new applications such as textiles and food packaging. Several research studies confirmed the remarkable activity of silver nanoparticles against microorganism [20].

The objectives of this study were to produce silver nanoparticles by the bark of *Acacia nilotica* extract as an inexpensive source to dye enhancer. Also, assay the antibacterial activity of the dyed cotton fabrics and the assessment of the *In-vitro* contamination control in the cotton plant tissue culture.

2. Experimental

1) Plant Extraction

Extraction of the Acacia bark dye was carried out according to the method explained by [21]. The optimum condition for the dye extraction was 0.30 M NaOH, Temperature 100˚C, Time 75 min and L: R (1: 12.5).

2) Preparation of Nano–sized sliver nanoparticles (AgNPs)

AgNPs were prepared according to the method previously explained [22,23] With minor

adaptation. The process carried out by adding extract of the acacia bark with 50 ml of $AgNO_3$ solution (ratio 2:1) then, the mixture was mixed with de-ionized water until to reach a final volume of 200 ml. This mixture was subjected to microwave irradiation for 3 min at 800 watt and then allowed to stand for 24 h. No additional reducing agent or surfactants were needed for the synthesis of AgNPs.

3) **Mordanting technique**

Cotton fabric samples were pre-mordanted with 1% of ferric chloride solutions at room temperature for 24 h with a liquor ratio of 1:30. After mordanting, the samples were rinsed in cold water to remove the excess of mordant and then dyed with dye extract.

4) **Dyeing procedure**

The cotton samples were dyed with Acacia bark dye extract carried out according to our previous research [24].

5) **Treatment of AgNPs on the Cotton Fabric**

Application of synthesized AgNPs on cotton was carried out according to the method explained by [24]. By dispersion of AgNPs to cotton fabric using exhaust method at 40°C and liquor to material ratio of 50:1. After 1 h, the temperature was increased to 80°C for 30 min. then the treated fabric samples were thoroughly washed and dried in air.

6) **Measurements**

Measurement of Color Strength and Color components

Color components of dyed fabrics were measured using a UV–VIS using the double beam spectrophotometer (Perkin-Elmer Company–USA, Model Lambda 35 [25]. The measurements of dyeing response (K/S), reflectance (R%) and total color difference (ΔE) were measured according to ASTM E313-96 using CIE color system coordinates, over the range of 400–700 nm. The reflectance value of a specimen for the wave length of 400nm–700nm with 10nm intervals was evaluated using spectrophotometer (Lambda 35, Perkin-Elmer, USA). The measurement was performed in accordance to [26]. Using CIE color system coordinates. Color strength (K/S) can be determined using this reflectance value into the KubelkaMunk's equation:

$$K/S = (1- R)^2/2R$$

Where, R=Reflectance of an incident light from the dyed material, K=Absorption, and S= Scattering coefficient of the dyed fabric.

7) **Measurement of Color Fastness Properties**

Color fastness properties of all dyed specimens were determined as according to [27].

8) **Particles Size Distribution**

The diameter and distribution of silver nanoparticles were calculated by 4 pi analysis software using TEM photos. The average diameter of the silver nanoparticles was determined from the diameter of at least 20 – 100 nanoparticles.

9) **Antibacterial Activity Assay**

Culture Media:

Nutrient agar was prepared by adding 15g of bacteriological agar to 25g of nutrient broth before adding 1 Liter of deionized water and autoclaved [28].

Bacterial Strains:

Escherichia coli and *Bacillus subtilis* were taken from frozen stocks and sub cultured into nutrient agar before being incubated at 37°C for 24 h then sub cultured twice more in the same manner before being used in the next experiment [29].

10) **The Disk Diffusion Assay**

The Disk Diffusion assay was demonstrated by [30,31]. For this experiment, the dyed cotton fabric variety Giza 86 by nano–sized silver particles (AgNPs) with metal mordant (ferric chloride) and without mordant were cut into 4 disks and placed on the same plate, Kanamycin disk (positive control), sterilized deionized water disk (negative control) and dyed cotton fabric disk using AgNPs. Then the disks were placed on top of the surface of the plates and incubated for 24 h at 37°C.

11) **Contamination Control Assay of Plant in vitro Cultures**

Plant Material

Plant material was obtained from 4 months old Egyptian cotton plant variety Giza 86 grown at the greenhouse of Cotton Research Institute, Agriculture Research Center, Egypt. Shoots with a length of 15–20 cm of the cotton plant were collected. Shoots were excised into 1.5–2.5 cm-long nodal segments.

Sterilization and Culture Initiation For surface disinfection, the explants were washed for 5 min in a solution of dishwashing liquid in 100 ml of water, then the explants were rinsed under running tap water for 1 h. Afterwards, they were soaked in the recommended concentration of 0.3 % (w/v) Rizolex-T 50%WP (fungicide) for 10 mins then washed thoroughly with sterile distilled water for 5 min. For internal disinfection, two separate experiments were conducted. In the first experiment, the explants were pre-sterilized by immersion in 70% ethanol for 60 s followed by a rinse with sterile distilled water. These pre-sterilized explants were exposed for 5 min as immersion time to the same prepared AgNPs solution (2:1) which used for the evaluation of cotton fabric dying process in this study in the previous application. To avoid phenolic contaminants, the explants were submerged in 0.5% citric acid for 3 min. Finally, they were cultured in jars containing [32], medium supplemented with 0.5 mg L^{-1} BAP, 30 g L^{-1} sucrose and 8gL^{-1} agar and labelled as MS1 medium. In the second experiment, the explants were treated with 50% (v/v) sodium hypochlorite for 5 min and 20% (v/v) sodium hypochlorite for 5 min. Then, the explants were washed three times with sterilized distilled water to remove all the traces of sodium hypochlorite. Then the explants were dipped in 0.5 % citric acid for 3 min. The designed experiments for sterilization were demonstrated in **Figure (1)**. Finally, they were cultured in the same jars of MS1 medium which used for the first experiment to compare the results of both sterilization experiments under the same conditions. The pH of all media was adjusted to 5.8 before autoclaving and then grown in a growth room with a light intensity of 2500–3000 lux, photoperiod of 16/8 h light/dark, the relative humidity of 45% and constant temperature of 25±1˚C. After 1 week, fungal contamination percentage, bacterial contamination percentage of cotton plant variety Giza 86 buds were recorded each day.

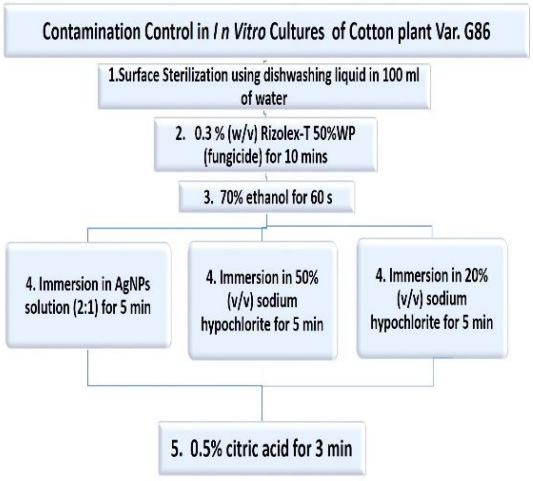

Fig. 1. The sterilization experiments of cotton plant Giza86 for in vitro cultures

3. Results and Discussion

1.UV-Vis absorbance study

The silver nitrate solution turned from yellowish to dark brown represent the formation of AgNPs as shown in **Fig. (2)**. The absorption peak at around 426 nm for AgNPs are characteristic of those nano-metal particles.

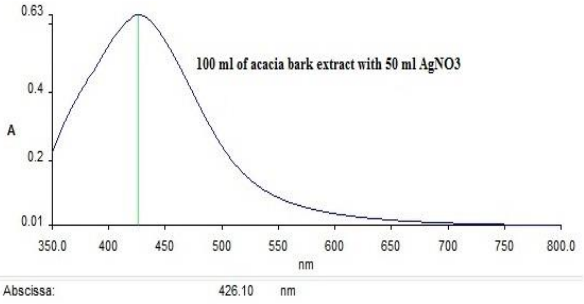

Fig. 2. Uv-Vis spectrum of nano Ag particles with the aqueous extract of acacia nilotica extracts

2. Characterization of the prepared AgNPs

Particle size distribution (PSD) is typically measured using laser scattering or diffraction techniques for any powders. In this technique, the 'halo' of diffracted light is measured on particles suspended in a liquid. Basically, the angle of diffraction increases as the particle size increases. **Fig. (3)** shows the particle size distribution of the prepared AgNPs by 4 pi analysis software using TEM photos. The results obtained showed that the

particle size was relatively non-uniform. It was observed that two peaks were recorded for measurement, where the major peak was recorded with an average particle size of 125.4 (96 % intensity) and a minor peak with an average particle size of 4466 nm (4% intensity). This occurrence of the two peaks might due to the orientation of rod-shaped AgNPs across the scattering light during measurement, or simply because of dirt. The result was in accordance with the results obtained by [24,33].

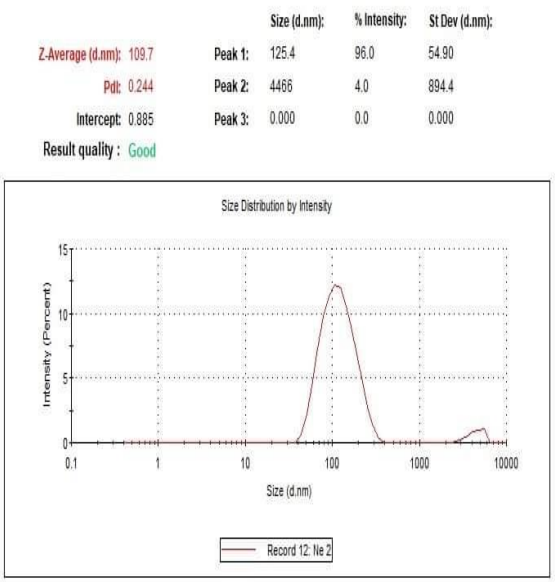

Fig. 3. The particle size of the prepared AgNPs

3. The zeta potential value of the AgNPs

Zeta potential (estimated as surface charge) is often measured by chase the moving rate of negatively or charged particles across an electrical field [34]. Typically a worth but –15mV represents the onset of agglomeration. Values larger than –30 mV usually signify that there's decent mutual repulsion which ends up in mixture stability. As shown in **Fig. (4)**, the letter potential worth of the AgNPs suspension was -7.76 mV attributed thereto the chemical bond shaped by electricity attraction of the Ag^{+} of the and also the NO^{3-} cluster might be broken.

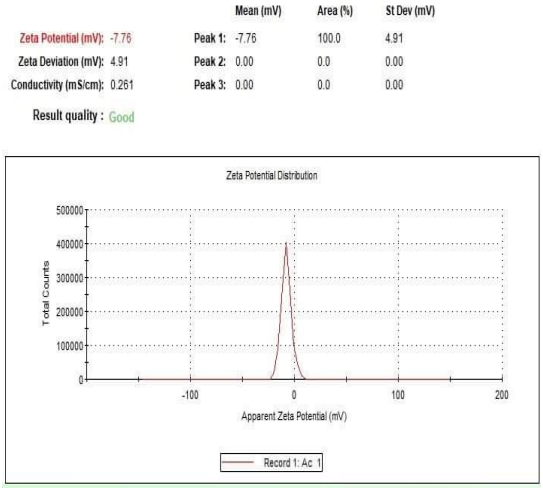

Fig. 4. the zeta potential value of the AgNPs

4. Color strength and color componentsof fabric dyed with and without (AgNPs)

Table (1) showed the CIE L*a*b* color system is widely used in the colormeasurement of textiles.In this system, L* shows the lightness of the fabric and a* and b* indicate red-green(redder if positive; greener if negative) and yellow-blue colors (yellow if positive; blue if negative), respectively. These data showed the effect of silver nanocomposite on the coated fabric coloration under washing process. Through increasing the amount of the silver nano - particles on the fabrics, b* values increased and the color of the fabrics tuned to creamy-yellow indicating the formation of the nanoparticles on the fabric surface. In contrast, L* values are decreased as the lightness of coated fabrics is decreased. However, by washing, b* values were decreased. By comparing the data measured for the fabric L* values were increased, as the lightness is increased by washing in samples dyed by acacia extract without using nano- sized sliver particles (AgNPs).These results agree with [35].

Table 1. Effect of dyeing process on K/S and color components.

Variety	Treatments	Color strength (K/S)	Color components		
			L*	a*	b*
Giza 86	Fabric dyed by Acacai without mordant	1.07	70.70	4.87	11.17
	Fabric dyed by Acacai with mordant (ferric chloride)	1.87	55.56	1.50	4.35
	Fabric dyed by Acacai with (AgNPs) and without mordant	1.23	66.92	4.51	10.09
	Fabric dyed by Acacai with (AgNPs) and with mordant (ferric chloride)	2.44	49.59	0.91	2.96

As shown, more than double amount of relative color strength (%) on dyed cotton fabric by acacia without mordant was obtained.This effect could be attributed with the presence of acidic colouring components such as quercetin, acacetin, tannins, gallic acid, etc, [26]. These acidic groups combined with acidic part of the coloring component and dragged as salt into the alkaline medium. It is also observed that relative color strength value in alkaline medium was much higher with acacia's bark in comparison to relative color strength value obtained with acacia bark with similar set of conditions. As known most of acidic tannins are present in acacia's bark..

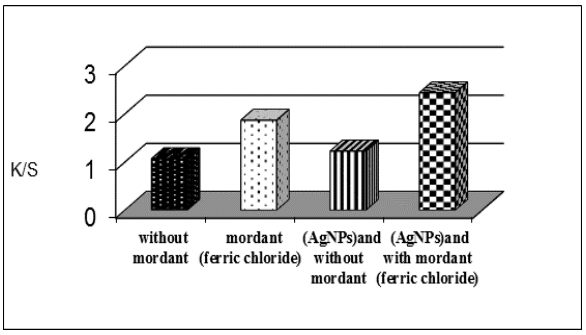

Fig. 5. Effect of dyeing by Acacia bark extract with and without mordant comparing to dying with the prepared AgNPs with and without mordant on color strength (K/S).

Table 2. Effect of dying process on fastness Properties.

Variety	Samples	Color Fastness to Washing		Color fastness to perspiration				Rubbing fastness		Light fastness
				alkaline		acidic		dry	wet	
		Color change	staining	Color change	staining	Color change	staining			Color change
Giza86	a.	4	2-3	3-4	3-4	3-4	3-4	4-5	3-4	4
	b.	4	3-4	3-4	3-4	3-4	3-4	4-5	3-4	5
	c.	5	4-5	4	4	4	4	4-5	4	5
	d.	5	4-5	4-5	4-5	4	4	4-5	4-5	6

a. Fabric dyed by Acacia without mordant.
b. Fabric dyed by Acacia with mordant (ferric chloride).
c. Fabric dyed by (AgNPs) without mordant.
d. Fabric dyed by (AgNPs) with mordant (ferric Chloride).

5. The fastness properties of the fabric dyed without and with (AgNPs)

The result is assessed in the usual way in term of the grey scale values for the staining of adjacent cotton material and change in the shade as well. The results indicated that the use of AgNPs was advantageous for the wash fastness in which shows 5 grey scales rating in comparison to sample dyed without AgNPs. Thus AgNPs treatment was proved to be better with reference to wash fastness. This could be attributed to the formation of the insoluble color complex on fabric. The higher grey scale rating could also be attributed to coordination complexes. Also, fastness to perspiration in alkaline and acidic was given high values (4-5) in dyed by acacia with AgNPs with mordant (ferric chloride). Light fastness values of fabric dyed with Acacia's bark extract with and without AgNPs are indicated in Table (3). The light fastness of dyed fabric increase with increasing of dye concentration, it gave value (6) in fabric dyed by acacia with AgNPs and with mordant, the main cause being an increase in the average size of submicroscopic particles in which the dyes form in the fabric because of the smaller area of dyes exposed to air and light. Although, the samples had shown better rubbing fastness properties in comparison to sample dyed without AgNPs due to the increased of dye uptake in dyed with AgNPs and with the mordant depth of shade was improve

6- Antibacterial Activity

The synergistic antibacterial activity of silver nanoparticles from *Accacia Nelotica* bark extract in both dyed samples of cotton fabrics with metal mordant (ferric chloride) and without mordant were investigated against two human pathogens *Escherichia coli* (Gram Negative) and *Basillus subtilis* (Gram-positive). For the comparison purpose, the standard kanamycin discs (30 mg/disc) were used. The result of antimicrobial activity was measured in term of the zone of inhibition (mm), with the evidence that the dyed sample of cotton

fabric with metal mordant (ferric chloride) has the highest activity against both organisms (gram-positive) bacteria *Basillus subtilis* and (gram-negative) bacteria *Escherichia coli*. The zones of inhibition can be seen in Table (4). The reports on the inhibitory action of silver ions on microorganisms show that upon silver ion treatment, DNA loses its replication ability and expression of ribosomal subunits proteins, as well as some other cellular proteins and enzymes essential to ATP production, becomes inactivated [36].

Table 4. The bacterial inhibition zones (mm).

Organism	Cotton fabric dyed by AgNPs with the metal mordant ferric chloride	Cotton fabric dyed by AgNPs without metal mordant
Escherichia coli	25 mm	20mm
Basillus subtilis	29mm	23mm

7- Contamination Control in Plant In Vitro Cultures.

Plant tissue culture techniques are needed for several academic studies, as well as to applied researches, different researches have been intensive on antifungal and antibacterial materials. Silver or silver ions have long been known to have acute inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. This research assayed the antimicrobial activity of nanosilver on in vitro cotton cultures and the ability of nano silver to reduce the microorganism. The prepared solution of AgNPs (2:1) was used for sterilization of cotton explants comparing to use two concentrations of sodium hypochlorite 20% and 50% (v/v) of sodium hypochlorite for 5 min. The experiments were illustrated in Figure (6). In the same jar, the treated explants were cultured on MS medium to be cultured under the same conditions. To define the site of each treated explant, the explants sites were marked on the bottom side of jars to facilitate and recorded the contamination observation.

The results showed that the immersion in AgNPs solution (2:1) for 5 mins had significant effects to eliminate the fungal and bacterial contamination in comparison to the usual method using both concentrations of sodium hypochlorite 20% and 50% (v/v) for 5 mins with no efficient in sterilization. Storage and handling of NaOCl need process safety procedures and containment to avoid exposure to workers, technicians, the environment and to prevent loss of potency through exposure to air, which causes it to deteriorate. Sodium hypochlorite is a common disinfectant that is used for sterilization of seeds and tissues in most studies even in plant biotechnological studies [37]. Noteworthy, It was observed that the prepared AgNPs solution (2:1) was efficient for controlling and eliminating the internal contamination of the woody plant (Valeriana Officinalis) using a submersing technique to surface sterilize explants. According to the obtained results in this study, the nanosilver synthesis from acacia nelotice extract will be an important method for the preparation of disinfectants of in vitro tissue cultures in future studies.

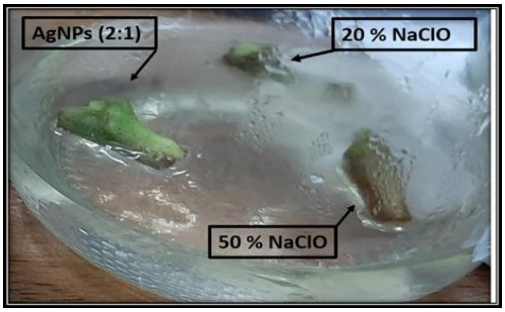

Fig. 6. The effect of AgNPs solution (2:1) on fungal and bacterial contamination in comparison to 20% and 50% (v/v) sodium hypochlorite for 5 min

4. Conclusion

This study concluded that the synthesis of silver nanoparticle using plant extract especially Acacia Nelotica has many advantageous more available, comparative, simple method for preparation in the laboratory with inexpensive price and less toxic nature in comparison to the other transition metals for making silver and its nanoparticles. The exploration of two different applications of the prepared silver nanoparticle using *Acacia Nelotica* extract is established in this paper. The dyeing of cotton fabric variety Giza 86 was evaluated and resulted the high values in all fabric tests with an efficient antimicrobial activity. Furthermore, The excellent antimicrobial activity of the prepared silver nanoparticle has been recorded in the new application for controlling the in vitro contamination of Egyptian cotton plant variety Giza 86 as the nanoparticles are found to act on different organisms with the significant elimination of the all contaminates in all treated explants confirming the possibility to apply silver nanoparticles for in vitro sterilization protocols in the future.

5. Conflicts of interest

The authors declare no conflict of interest.

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