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Honey Bee for Eco-friendly Green Synthesis of Silver Nanoparticles and Application to Cotton Textile

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A RAPID and eco-friendly method was devised for fabrication of sliver nanoparticles AgNPs at low temperature using honey bee as a reducing agent for silver nitrate and stabilizing agent for AgNPs formed thereof. Formation of AgNPs was evaluated by monitoring UV-vis spectra of the silver colloidal solution, whereas the size of the formed nanoparticles were measured by transmission electron microscopy(TEM). Different reaction parameters pertaining to the synthesis of AgNPs were studied in order to establish optimization. Accordingly, by the most appropriate conditions for preparation of AgNPs colloidal solution having adequate concentration for industrial application, that is, (1080 ppm) were: 10 ml honey bee, 0.17g $AgNO_3$/ 100ml, pH11, temperature 50^oC and reaction duration of 60 min. Cotton fabric was treated by the prepared silver nanoparticles colloidal solution using pad-dry curing technique in presence and absence of a binding agent. Thus treated fabrics were evaluated using scanning Electron microscopy (SEM) and bio-assay for antimicrobial activity.

Keywords: Honey bee, Nanoparticales, Antimicrobial activity and Cotton fabric .

Honey bee provides salient health such as antibacterial, antioxidant, antiflamatory and antitumor [(1)]. That is why it has found widespread utilization as functional food ingredient and therapeutic agent for a very long time.

Honey is a sweet viscous fluid produced from bees , and is mainly composed of carbohydrates, enzymes, vitamins, minerals and antioxidants[(2)]. The major constituents of honey are fructose and glucose and it contains aminoacids that help up calcium in the body. Honey has been subjected to extensive study all over the world on its ingredients, physicochemical properties, vitamins, mineral content and quality control. Honey is rich in vitamin C and the important minerals present are K and Mg [(3)] .

Recently, honey has been used in the field of nanotechnology to benign synthesis of nanoparticles[(3,4)]. Green synthesis strategy for AgNPs from honey is developed due to increasing awareness about environment. The use of non-toxic

chemicals, environmentally eco-friendly and renewable materials are some of the key issues that should be considered in such strategy [5] .

Large nanomaterials have wide-ranging implication in a variety of areas, including physics, chemistry , electrics, material science and the biomedical science[6]. Among these AgNPs form exhibit strong cyctotoxicity towards a broad range of microorganisms and their use as an antibacterial agent is well known[7].

It should be emphasized, however, that most of the synthetic methods reported to date rely heavily on the use of organic solvents and toxic reducing agents like hydrazine [8],N,N-dimethly formamide [9] and sodium borohydride[10,11]. All these chemicals are highly reactive and pose potential environmental and biological risks.

Current work is undertaken with a view to establish an innovation which forms the base for rapid and eco-friendly method for synthesis of AgNPs. The unique feature of the innovation is the use of honey bee which is nontoxic, biodegradable and biocompatible agent. Meanwhile it plays dual action: a) as a reducing agent to convert silver ions to nanosilver metal and b) to stabilize the formed AgNPs by capping and/ or encapsulating them thereby preventing their aggregation. Another uniqueness of the innovation in question is the low temperature at which the synthesis of AgNPs is carried out. UV-visible absorption spectroscopy and transmission electron microscopy (TEM) are used for characterization of AgNPs. Also, investigated is the application AgNPs colloidal solution to cotton fabrics and evaluation of the treated fabrics using scanning electron microscope (SEM) and antimicrobial activity.

Experimental

Materials and methods

Natural honey bee was obtained from native hives honey . Silver nitrate ($AgNO_3$ 99%) and sodium hydroxide were from sigma-Aldrich\Germany. Binder (printofix binder MTBEG liquid (based on acrylate) was supplied by Clariant Cairo, Egypt. Mill desized, scoured and bleached cotton fabrics were supplied by EL-Nasr Company for Spinning , Weaving and Dyeing, EL-Mehallah El-Kubra, Egypt.

Synthesis of silver nanopartiles (AgNPs)

AgNPs were prepared as per the devised method as follows. A known weight of honey bee was dissolved in 95ml distilled water and the pH of the solution was adjusted at certain pH within the pH range of 8-11 using dilute solution of sodium hydroxide. The solution was completed to 100 ml by distilled water then brought to heating at different temperatures for some minutes .At this end different amounts of already prepared $AgNO_3$ solution were added dropwise under the action of magnetic stirrer. Factors affecting the reduction efficiency and stability as well as shape and size of the formed nanoparticles were studied as given later.

Characterization techniques of silver nanoparticles

Ultravoilt-visble (UV-vis) spectra

UV-vis spectra have been proved to be quite sensitive to the formation of silver colloids because AgNPs exhibit an intense absorption peak due to the surface Plasmon excitation which describes the collective excitation of conductive electrons in a metal. AgNPs embedded in honey were recorded in spectra 50 ANALYTIKA JENA SPECTROPHOTOMETER from 300 to 550. Distilled water was used as the blank.

Transmission electron microscopy (TEM)

Shape and size of AgNPs were practically obtained using TEM; JEOL-JEM-1200. Specimens for TEM measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of 100 nanoparticles found in several arbitrarily chosen areas in enlarged microphotographs.

Results and Discussion

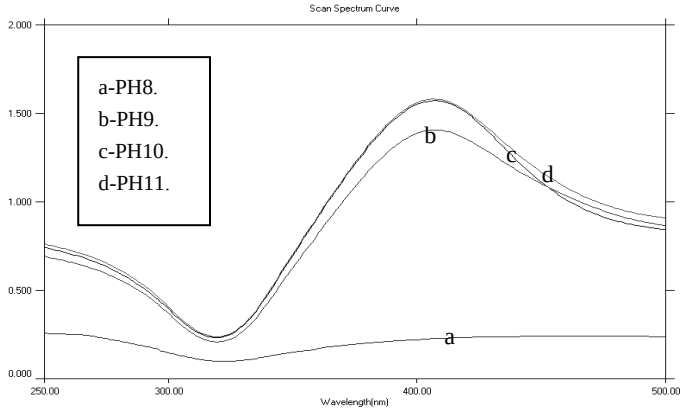
Honey contains reducing agents that may be exemplified by fructose, glucose and vitamin C. Reduction of Ag ions takes place on the addition of sodium hydroxide to honey solution. The base facilitates the opening of the glucose ring by the abstraction of the α-proton of the sugar ring and the metal ions oxidize glucose to gluconic acid [(4)]. It is also possible that sucrose and proteins/enzymes play a role in the reduction. With this in mind, factors affecting the formation of AgNPs from $AgNO_3$ under the reducing activity of honey are present under.

pH of reaction medium

1ml of honey bee was dissolved in 95 ml distilled water using heating magnetic stirrer. After complete homogeneity of honey with water the pH was adjusted to 8,9,10 or 11 using dilute sodium hydroxide meanwhile the temperature was adjusted at 50^{o} C. At this end ,silver nitrate(0.1N) was added dropwise (1ml each) until a total volume of the reaction medium of 100 ml was reached.

Figure 1 shows the UV-vis spectra of the silver colloid synthesized at pH 8,9,10 or 11 using $AgNO_3$ in presence of honey as reducing and stabilizing agent. Obviously ,at pH 8 the electronic absorption spectra is very broadened without any absorption intensity of the Plasmon peak around 405 nm. The implication of this is that at pH8, the silver ions could not be converted to silver nanoparticales under the conditions used. In contrast carrying out the synthesis of AgNPs at pH9 verifies the reduction of Ag^{+} to Ag^{0} as evidenced by the appearance of Plasmon peak at 415nm which, in turn, signifies enhancement in the absorption intensity and slitting the band toward longer wavelength. Similar absorption bands but with greater intensity are observed when the silver nanoparticales were synthesized at pH10 or 11. It may be, therefore, concluded that the formation of AgNPs is directly related to the pH of the reaction medium. AgNPs can only be formed with greater intensity at pH 10 or 11. Indeed pH's

lower than pH9, fail completely to accomplish such nano formation under the conditions outlined above.

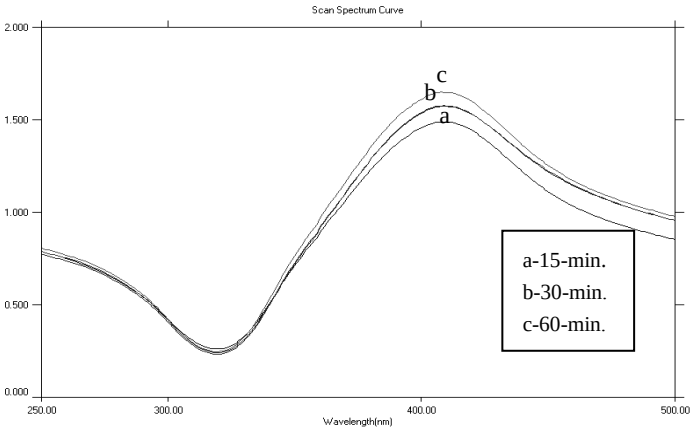


Reaction conditions: Honey, 1ml and $AgNO_3$, ، 0.1 m mole/100 ml water ، temp 50 °C; time 60 min.

Fig. 1. Eeffect of pH on the formation of silver nano particales as monitored by UV-vis spectroscopy.

Reaction duration

Figure 2 shows the UV-vis absorption spectra of AgNPs colloidal solution prepared under the said conditions for different durations (15,30,60 min) . As is evident, formation of AgNPs occurs regardless of the magnitude of duration used. Nevertheless, the Plasmon intensity of the absorption peak at 415nm is greater at longer than shorter duration within the range studied. It is understandable that as the duration is prolonged, better opportunities are given for reactants to contact and undergo reactions yielding ultimately AgNPs with greater intensity.

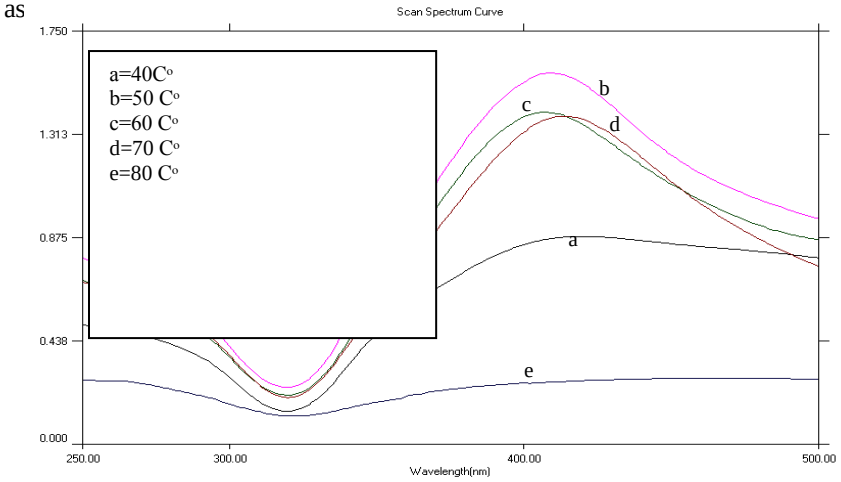


Reaction conditions: Honey, 1ml and $AgNO_3$, ، 0.1 m mole/100 ml water ، temp 50 °C; pH11.

Fig. 2. Effect of duration on the formation of silver nano particales.

Temperature of the reaction medium

Figure 3 shows the UV-vis absorption spectroscopy of AgNPs prepared at different temperatures (40,50,60,70 and 80^{o}C) at pH 11 for 60 min. As is evident the temperature plays a key role in both the reduction reaction and AgNPs formation and size. At 40^{o}C, the absorption intensity of the Plasmon peak is very broad around 400 nm indicating lower conversion of Ag^{+} to Ag^{0}. This peak becomes stronger and narrower at 410nm by carrying out the AgNPs synthesis at 50^{o}C.This means higher conversion of Ag^{+} to Ag^{0} with smaller size nanoparticles. Similar situation is encountered with respect to 60^{o}C and 70^{o}C. On the other hand, when the synthesis of AgNPs was performed at 80^{o}C, the absorption intensity of the Plasmon peak disappears, signifying the no existence of AgNPs. It is likely that the honey along with its reducing constituents undergo decomposition and in so doing, lose their role as

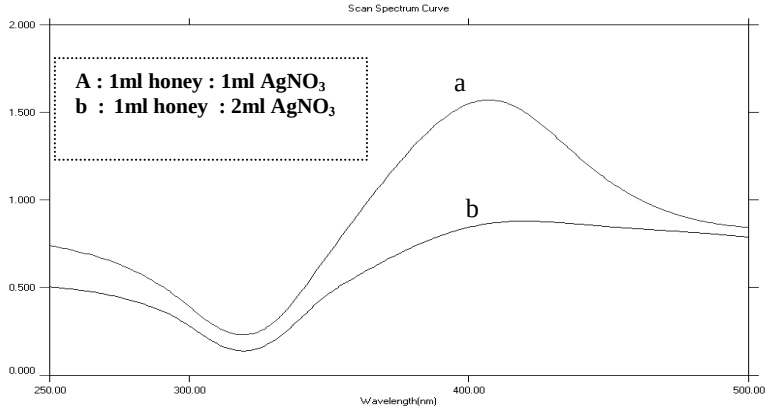


Reaction conditions: Honey, 1ml and $AgNO_3$, ، 0.1 m mole/100 ml water ; pH11.; time 60 min.

Fig. 3. Effect of temperature on the formation of silvr nano particales.

Silver ion concentration

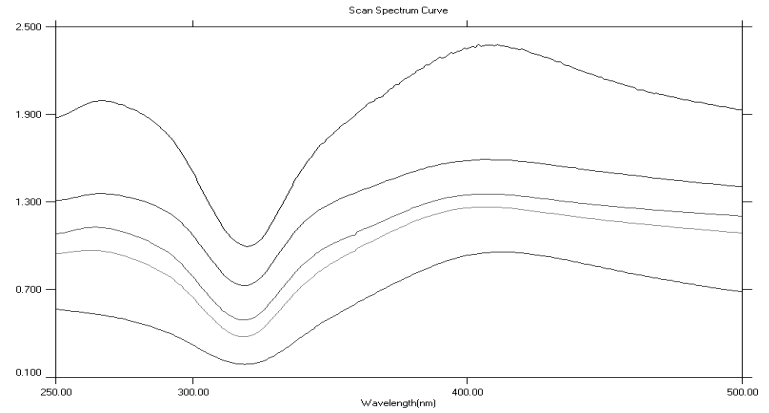
Figure 4 shows the UV-vis spectra of AgNPs which were brought about by independently incorporating two different amounts of $AgNO_3$ (100ppm and 200ppm) in a solution containing constant concentration of the honey (1ml). It is clear that AgNPs accomplished through the use of $AgNO_3$ at concentration of 100ppm exhibit an absorption peak at wave length 405nm; the intensity of this Plasmon peak is much greater than that obtained using $AgNO_3$ at a concentration of 200ppm. This clearly advocates the use of lower concentration of $AgNO_3$ which, indeed favors formation of AgNPs with smaller size and better stability. The use of higher concentration of $AgNO_3$ seems to produce AgNPs with larger size by virtue of their aggregation most probably owing to lower stability. Hence, in order to induce efficient reduction and better stability along with extremely small-sized silver particles, certain ratio of silver nitrate to honey in the reaction medium must be established as detailed below.



Reaction conditions: Honey ,1ml,temp.50^{o}C and time 60 min.

Fig. 4. Effect of silver ion concentration on AgNPs sysnthesis .

Figure 5 depicts the UV-vis spectra of AgNPs resulting from the use of different $AgNO_3$:honey ratios. In these ratios the concentration of $AgNO_3$ ranges from 100 to 1000 ppm whereas the honey concentration ranges from 1 to 10ml. The dilution for the measurements was 20 times dilution. Obviously Fig. 5 reveals that similar absorbance spectra are obtained at wavelength 405 nm with marginal increase in the intensity of the absorption peak by increasing the amount of $AgNO_3$ and honey in their ratio incorporated in the reaction medium. The figure displays also that the UV spectra characterizing the peak of silver ion disappear.



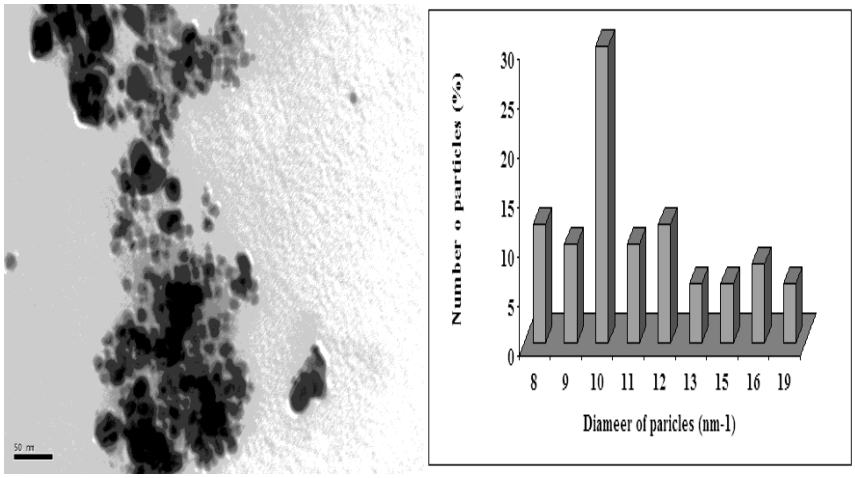
HONEY:$AgNO_3$
a) 1ml:.100ppm
b) 2ml:200ppm
c) 4ml:400ppm
d) 8ml:800ppm
e) 10ml:1000ppm

Reaction condition**:** Temp 50 ^{o}C; pH 11 and time 60 min.

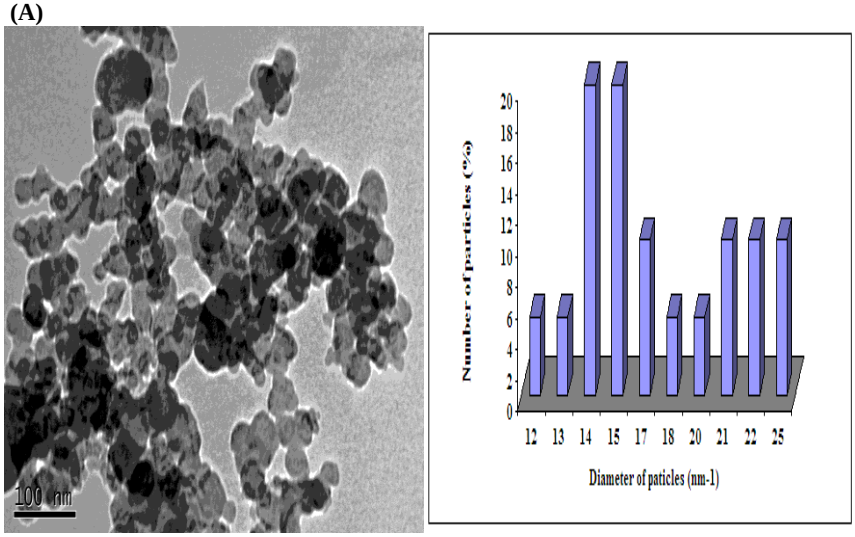
Fig. 5. Effect of honey: silver nitrate ratios at different concentrations on the foration of silver nano particales .

Figure 6a illustrates the TEM image and the particle size distribution histograms of silver nanoparticles (100ppm)formed after 60 min using honey bee at concentration of 1ml. The TEM image shows small size spherical particles. The corresponding size distribution histogram clearly illustrates the size of the formed particles. The majority of which attained 10nm.

Figure 6b shows the TEM image and particles size distribution histograms of silver nanoparticles(1000ppm) formed using 10 ml honey bee for 60 min. The size of the majority of the formed particles ranges between 14-15 nm.



(A)



(B)

Fig. 6. Representative TEM images and histograms of particles size distribution of Ag-NPs (A :100 ppm and B: 1000 ppm) prepared by honey.

SEM micrographs for cotton fabrics treated with AgNPs

The interaction between fibers and AgNPs involves Physical adsorption on the fabric surface[11,12].

Figures 7 (a-c) shows the scanning electron microscope (SEM) images of cotton fabrics before and after treatment with AgNPs at two different concentrations (50ppm,100ppm).It is seen that the untreated fabric is characterized by smooth surface without any disturbed structure (Fig. 7a). On the other hand, treated fabrics contain homogeneous deposition of AgNPs (Fig. 7b and c). This is the case when the fabrics were treated with solutions containing 50 and 100 ppm of AgNPs. However the amount of AgNPs deposited on the fiber surface of cotton fabrics increases by increasing the concentration of AgNPs colloids solution from 50 to 100 ppm.

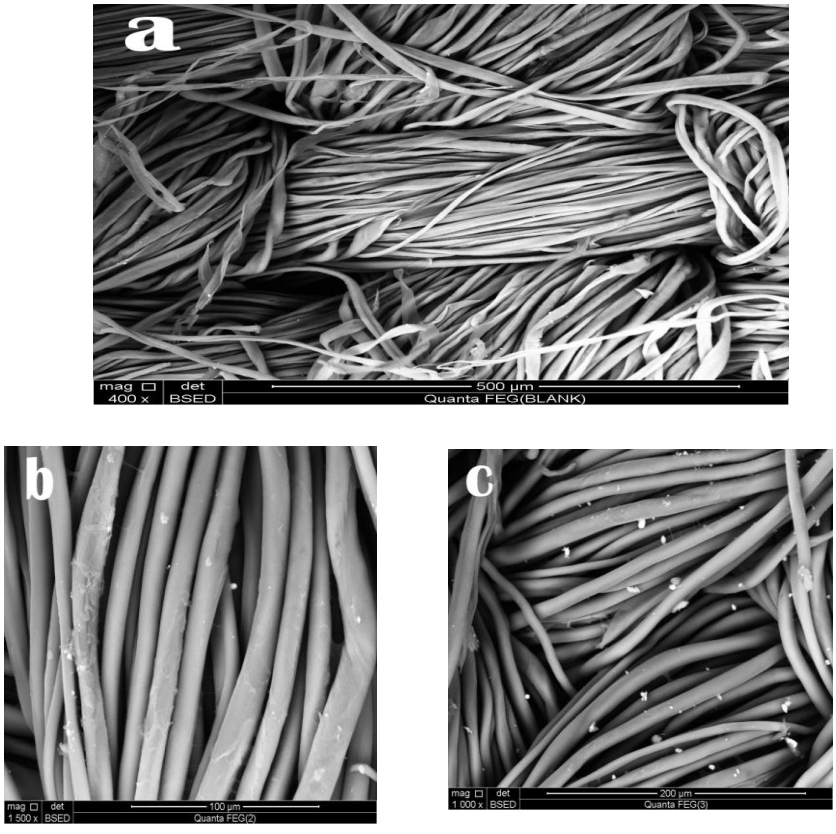


Fig. 7. SEM image of : (a) untreated cotton fabric, (b) nanosilver particles on cotton using 50 ppm ،(c) nanosilver particles on cotton using 100 ppm.

Antibacterial activity of cotton fabrics treated with the synthesized AgNPs

Tables 1 and 2 disclose the antibacterial properties (bacterial reduction) of fabric treated with AgNPs colloidal solution with and without binding agent.

TABLE 1. Effect of washing on the antibacterial properties of honey bee unloaded and loaded with silver nanoparticale treated cotton fabrics without binder.

Washing	Bacterial reduction (%)					
	Nano-sized silver colloids concentration (ppm)					
	Honey bee unloaded with AgNP		50ppm		100ppm (Honey bee loaded with AgNPs)	
	S.aureus	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*
Before washing	70%	70%	100%	100%	100%	100%
After washing	60%	58%	86%	80%	87%	82%

The finishing bath formulation: a wet pick up of 100%, drying at 80°C for 3 min and curing at 140°C for 2min.

TABLE 2. Effect of incorporation binder with unloaded honey bee with AgNPS and loaded with 50ppm silver nanopartical solution on antibacterial properties of treated fabrics.

Washing	Bacterial reduction (%)			
	S.aureus	*E.coli*	*S.aureus*	*E.coli*
	(Honey bee unloaded with AgNPs)		(Honey bee loaded with AgNPs)	
Before washing	82%	80%	100%	100%
After washing	70%	68%	98%	96%

The finishing bath formulation**:** a wet pick up of 100%,1% a binding agent ,drying at 80°C for 3 min. and curing at 140°C for 2min.

Table 1 shows the effect of antibacterial properties of cotton fabrics treated with finishing bath containing honey bee unloaded with AgNPs and loaded with 50 and 100 ppm AgNPs in absence of binder. The treatment was carried out by padding the fabric in the finishing bath to a wet pick up of 100%, drying at 80°C for 3 min and curing at 140°C for 2min. Results (Table 1) signify that treatment with honey bee unloaded with AgNPs leads to reduction in the antibacterial colonies to a value of 70% before washing against *S.aureus* and attained to 60% after 10 washing cycles against *E.coli*. Regardless of the concentration of honey bee loaded with AgNPs used for the treatment, the reduction of bacterial colonies

attain a value of 100% against *S.aureus* as well as *E.coli* for the unwashed AgNPs-treated samples . Subjecting the treated fabrics to 10 washing cycles leads to values that slightly exceed 80% indicating a decrement in the reduction of bacterial colonies.

Table 2 shows the effect of incorporation of a binder (printofix Binder MTB EG liquid 1%) in the finishing bath formulation on the antibacterial properties of the treated cotton. The results of Table 2 illustrate that a) Reasonable improvement in the antibacterial reduction when 1% binder incorporated in the finishing bath containing honey bee unloaded with AgNPs compared with that in absence of the binder (Table 1). b) The fabrics treated with a solution containing AgNPs at concentration 50 ppm in the presence of binder exhibit excellent antibacterial properties. Bacterial reduction amounts to 100 % in case of *S. aureus* and *E.coli*. After 10 washing cycles, fabric samples display bacterial reduction of 98% and 96% reflecting the significant role of the binder in the fixation of AgNPs deposits within interior of the fibers of the cotton fabrics.

Conclusion

Green synthesis of silver nanoparticles could be accomplished using honey bee without any other reducing agents. Honey bee acts as a reducing agent for silver ion of $AgNO_3$and stabilizer for the AgNPs formed thereof. Preparation of a well-stabilized AgNPs solution with a concentration of 1000 ppm with a nono-sized particle a diameter of 14-15 nm could be realized.

The so obtained AgNPs were successfully applied to cotton fabrics. The use of 50ppm or 100ppm of AgNPs in fabric treatment is quite enough to exhibit excellent antibacterial activity against *E.coil* and *S.aureus*. The SEM micrographs and analysis reveal that the AgNPs are well dispersed as ultrafine deposits on the cotton fibers. A binder was successfully used to retain and for keeping antibacterial efficiency of the treated cotton fabrics. Indeed, current treatment is considered by all means safe, cost effective , eco-friendly and innovative process for fabrication of antibacterial finishing agent which can be used as finishing of cotton textile.

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استخدام عسل النحل بطريقة آمنة بيئياً لتحضير دقائق الفضة

منال البيسى ، هناء محمد حسين الرافعى* ، محمد حسين الرافعى وعلى حبيش
شعبة بحوث النسيج و* شعبة بحوث الصناعات الدوائية والصيدلية – المركز القومى للبحوث – الجيزة – مصر.

يعتبر عسل النحل من أهم المواد الغذائية الغنية بالسكريات المفيدة للإنسان منذ زمن بعيد حيث أنه يتكون أساساً من عديد السكريات ، الأنزيمات ، الفيتامينات والأملاح المعدنية بالإضافة إلى أهميته الغذائية فإنه يستخدم كمادة علاجية مقاومة للبكتيريا والأكسدة والالتهابات والخلايا السرطانية .

تهدف هذه الدراسة إلى استخدام عسل النحل كمواد آمنه بيئياً لاختزال نترات الفضة وتثبيت دقائق الفضة النانو مترية المتكونة حيث يتم تتبع سير التفاعل بإستخدام تحليل الموجات فوق البنفسجية المرئية وتقييم حجم الدقائق النانو مترية بإستخدام جهاز الميكروسكوب الإلكتروني النافذ .
وأوضحت النتائج أن أنسب الظروف هي :
1- درجة حرارة التفاعل 50 م .
2- زمن التفاعل 60 دقيقة .
3- الأس الهيدروجيني 11.
4- نسبة العسل إلى الفضة 10 سم 0,17:3 جم.

وذلك لإنتاج منتج بتركيز 1080 جزء في المليون صالح للتطبيق على المستوى الإنتاجي وتم معالجة الأقمشة المحتوية على السليلوز بمعلق الفضة النانو مترية وكذلك تم تقييم الأقمشة المعالجة بإستخدام الميكروسكوب الماسح الإلكتروني بالإضافة إلى خاصية مقاومة الأقمشة المعالجة للبكتيريا