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SCHOLARENA

Journal of Nanoscience and Nanotechnology Applications

RESEARCH ARTICLE

ISSN: 2577-7920

Fabrication of Albumin/Ag Nanoparticles by Using Ultrasonic Irradiation and Estimation of their Influence on Cell Division and Cytotoxicity of Allium cepa Roots

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Citation: Randa Fawzi Elsupikhe, Tahani Soliman Alfazani, Fawzia Muftah Al-Jazwia, Rasha M Sheltami (2021) Fabrication of Albumin/Ag Nanoparticles by Using Ultrasonic Irradiation and Estimation of their Influence on Cell Division and Cytotoxicity of Allium Cepa Roots. J Nanosci Nanotechnol Appl 5: 104

Abstract

Silver nanoparticles (Ag-NPs) have an enormous medical applications as biological engineering. Many methods have been used for the synthesis of Ag-NPs such as a chemical methods, but these methods have many of disadvantages that because most of the chemical which have been used for synthesis the nanoparticles are expensive and toxic, that, responsible for various biological risks. In this work, the green methods (ultrasonic- irradiation) for synthesis Ag-NPs have been used for solving these problems and Albumin has been used as a stabilizer to prevent the agglomeration between the NPs. Parameters such as the time of ultrasonic irradiation, concentration of $AgNO_3$ and concentration of Albumin have been optimized. The influence of AgNPs on cell division and cytotoxicity has been investigated using root tip cells of *Allium cepa* as an indicator organism. As a result from this study the Ag NPs could penetrate plant system and may impair stages of cell division, causing decreased mitotic index (MI) and increase in chromosomal abnormalities were observed in higher treatments of Ag Nps and at different time periods; bridge, stickiness, disturbed metaphase (CM), disturbed anaphase, laggards and fragments.

Keywords: AgNPs; Ultrasonic Irradiation; Cell Division; Cytotoxicity; Allium Cepa

Introduction

Recent years have perceived unprecedented development of research and applications in the area of nanoscience and nanotechnology. Nanotechnology defined as design, characterization, and application of materials whose smallest functional organization in at least one dimension. Nanoparticles, a unique subset of the broad field of nanotechnology, include any type of particle with at least one dimension of less than a 100 nanometers [1, 2]. Amongst of nanoparticles Silver nanoparticles (AgNPs), AgNPs are increasingly used in various fields, such as medical, health care, consumer, food and industrial purposes, due to their unique chemical and physical properties. These include electrical, biological properties and optical, and thermal [3]. Several methods have been developed for synthesis AgNPs including chemical methods [4] and green methods [5], physical methods as a green method for synthesis AgNPs such as: gamma ray radiation [6], laser ablation [7], microwave irradiation [8], Ultrasonic irradiation [9] and photochemical reduction [10]. Ultrasonic irradiation is one of the eco-friendly method for synthesis AgNPs including low-cost. The ultrasonic irradiation may affect the size distribution of the nanoparticles in a reaction times narrower range, the chemical effects of ultrasonic irradiation are due to the very high temperature and pressure that resulting from the broken of cavitation bubbles caused by ultrasonic irradiation [11,12]. However, for synthesized a stable nanoparticles plant extracts and natural polymers or protein may use as reducing and capping agents [13]. The Egg white protein or albumin is the liquid that surrounds the yolk of an egg besides his high food quality, Albumin has another advantage, is his solubility in water and tends to associate with metal ions in solution which combined with egg white to obtain novel nanomaterial with interesting properties [14]. Plant systems have a variety of well-defined genetic endpoints including alterations in ploidy, chromosomal aberrations, and sister chromatid exchanges. The Allium root chromosomal aberration assay is an established plant bioassay validated by the International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an efficient and standard test for the chemical screening and in situ monitoring for genotoxicity of environmental substances. Allium cepa has been used for evaluating chromosomal aberrations since 1920s.In the current paper, we report a simple and eco-friendly green way to prepare a stable AgNPs by using egg white as a stabilizer and ultrasonic irradiation as a reducing agent. Beside of that, estimate influence of AgNPs on cell division and cytotoxicity of *Allium cepa* roots [15].

Experimental

Materials

Albumin egg powder, $AgNO_3$, HCl, glacial acetic acid and absolute ethanol were supplied by Sigma–Aldrich as powder material and used without further purification.

Methods

Fabrication of Silver/Albumin nanoparticles AgNPs

Briefly, 100 ml of $AgNO_3$ solution (0.1M) was added to 400 ml of Albumin solution 0.1% (w/v). The solution was stirred 1 h to obtain $AgNO_3$/Albumin. The samples were exposed to high-intensity ultrasonic-irradiation at room temperature, by using the ultrasonic irradiation bath, for different time**.** Moreover, for investigation of the role of silver ions and Albumin in particle size of Ag-NPs, the experiment was also applied at different concentrations of silver nitrate and Albumin.

Cell Division and Cytotoxicity Experiment

Sterilized seeds were germinated in petri dishes lined with filter paper given renewed distilled water supply every 24 h for 4 days. Seedlings with 0.5 - 1 cm roots were treated with different Ag NPs concentrations (0, 0.0375 and 0.15 M) for different time periods (3, 6, 12 and 24 h). Then roots were fixed in freshly prepared Carnoy's solution (1:3 glacial acetic acid and absolute ethanol v/v for 24 h), then preserved in 70% ethanol until used. Roots were hydrolyzed in 1N HCl for 10 minutes at 60^0C in water bath. The roots were

washed with distilled water, then stained with 1% Acetocarmine for one hour (Sen and Kar, 2005). One root per slide was squashed and then examined under (XSZ-107 BN) microscope at 400X magnification. Three replicas were examined for calculating the mitotic index (MI) and percent of chromosome aberrations in dividing and non-dividing cells for each concentration and treatment times. Also, the seedlings treated with 0, 0.0375 and 0.15 M for 24 h were washed and for 72 days of recovery over distilled water. After 72 h of recovery, seedling lengths were measurement.

Characterization

UV/Vis spectrophotometer

UV-Vis is the one of the most important spectroscopic methods used for metal nanoparticles characterization. That because of unique surface plasmon resonance (SPR) shown by specific metal and metal oxide nanoparticles (Au, Ag, Cu, and Pt), the use of the technique is essential for early detection of nanoparticles presence. In this work, a UV-visible spectrophotometer [UV 1650 PC-Shimadzu B (Shimadzu Osaka, Japan)] was used in detection of SPR bands of nanoparticles obtained from the synthesis. About 70 µL of the sample was poured into cuvette. The spectra were run in the range of 200 to 800 nm.

Statistical Analysis

The results are expressed as mean values ± standard deviation (SD). LSD multiple range tests were used to compare the mean of the treatments at $P<0.05$. The significance of the difference between mean values was determined by two way analysis of variance.

Result and Discussion

Seven aqueous samples containing 0.1 M $AgNO_3$ and 0.1% albumin are irradiated by ultrasonic irradiation at different time. $AgNO_3$/Albumin is a colourless suspension, after applying ultrasonic- irradiation the colour of the prepared samples gradually changed from colourless to light brown to dark brown as shown in Figure1, that is indicative the formation of Ag-NPs in Albumin suspension [16]. 0 min denotes the $AgNO_3$/Albumin suspension without any irradiationand0, 20, 40, 60, 80, 100 and 120 min of ultrasonic- irradiation. The change in the colour depends on the increase of irradiation time.

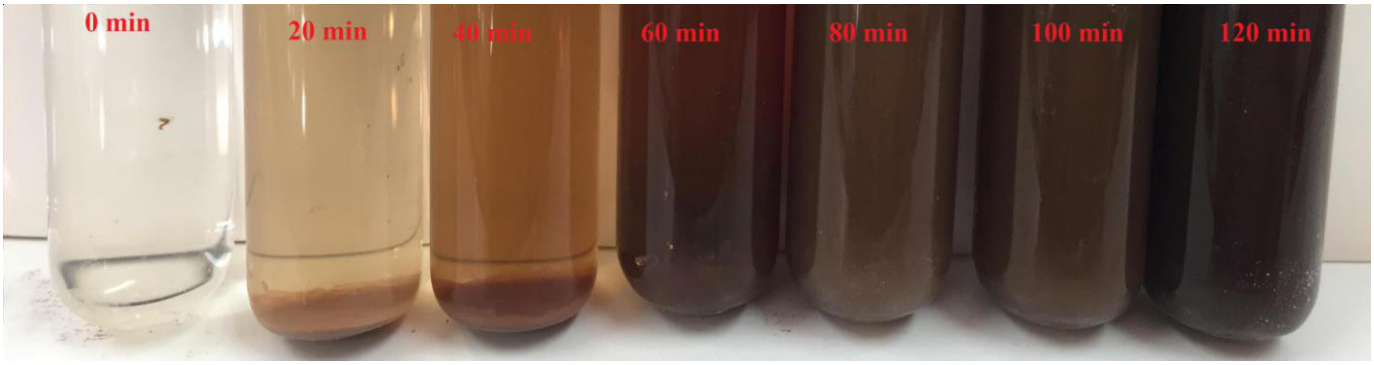

Figure 1: Picture of $AgNO_3$/Albumin (0 min) and Ag/Albumin (20-120 min) suspensions at different ultrasonic irradiation times.

The formation of Ag-NPs was identified by the (SPR) of $AgNO_3$/ Albumin and Ag in Albumin in the range of 300–800 nm. The SPR bands are influenced by the size, shape and morphology of the prepared nanoparticles [17]. Figure 2 (A) has shown the preparation of Ag-NPs in Albumin using different ultrasonic- irradiation times. The characteristic silver SPR band was detected at around 400-450 nm which indicated the formation of Ag-NPs [18]. As shown in Figure 2 before irradiation the solution by ultrasonic irradiation, there was no absorption which that means, there are no Ag-NPs in the solution. However, after irradiating the solution at different times of ultrasonic irradiation 0, 20 ,40,60, 80, 100 and 120min (b-g) respectively, the absorption bands are observed. The intensity of the SPR band also increased as the time increased which indicative that, the concentration of Ag-NPs increased as well [19]. In order to study the effect of Ag^+ concentration on Ag-NPs preparation, samples containing 0.05 to 0.25 M of $AgNO_3$ with constant

concentration of albumin (0.1%) are prepared and irradiated for 100 min. However, when increased the concentration of $AgNO_3$ to (0.1, 0.15 and 0.2M) (b, c and d), the absorbance displaying to lower wavelength (a blue shift), as shown the results in Figure 2 (B) when the initial Ag^+ concentrations are improved, Ag-NPs with smaller sizes and higher yields are produced [20]. Furthermore, when the concentration of $AgNO_3$ was increased to 0.25 M (e) the intensity of the absorption band was decreased, this refers to agglomeration that was happening in the solution [21].

As the Albumin concentration is varied from 0.05 to 0.25 % (a to e) the positions of the SPR bands characteristic of Ag-NPs was identified around 450 to 470 nm, which indicates the formation of Ag-NPs [22]. As shown in Figure 2 (C), when the concentrations of Albumin were increased 0.05, 0.1, 0.15, 0. 2, and 0.25 %, the intensity of the SPR peak also regularly increased. The increase of the absorbance was indicative that the concentration of Ag-NPs increased [23]. Furthermore, Figure 2 (c) shows that with an increase in the concentrations of Albumin, the absorbance also increased and shifted to lower wavelength to blue-shift, which referred to a decrease in the particle size [24]. Also, Figure 2 (c).was observed that the 0.2 and 0.25 % Albumin solution had a larger absorbance compared to other samples [24].

The size of Ag nanoparticles was calculated from the equation (1) [25], which, the size of Ag-NPs that synthesis by using ultrasonic irradiation After optimized, the time of irradiation , concentration of $AgNO_3$ and concentration of Albumin (ultrasonic irradiation for 100 min, $AgNO_3$=0.2 M and albumin =0.25%) were 3 nm.

$$r(nm) = \frac{-0.3049 \pm \sqrt{-26.23012 + (10240.72/\lambda(nm))}}{-6.3829 + (\frac{2483.2}{\lambda(nm)})} \quad (1)$$

Where λ is the maximum Absorbance wavelength.

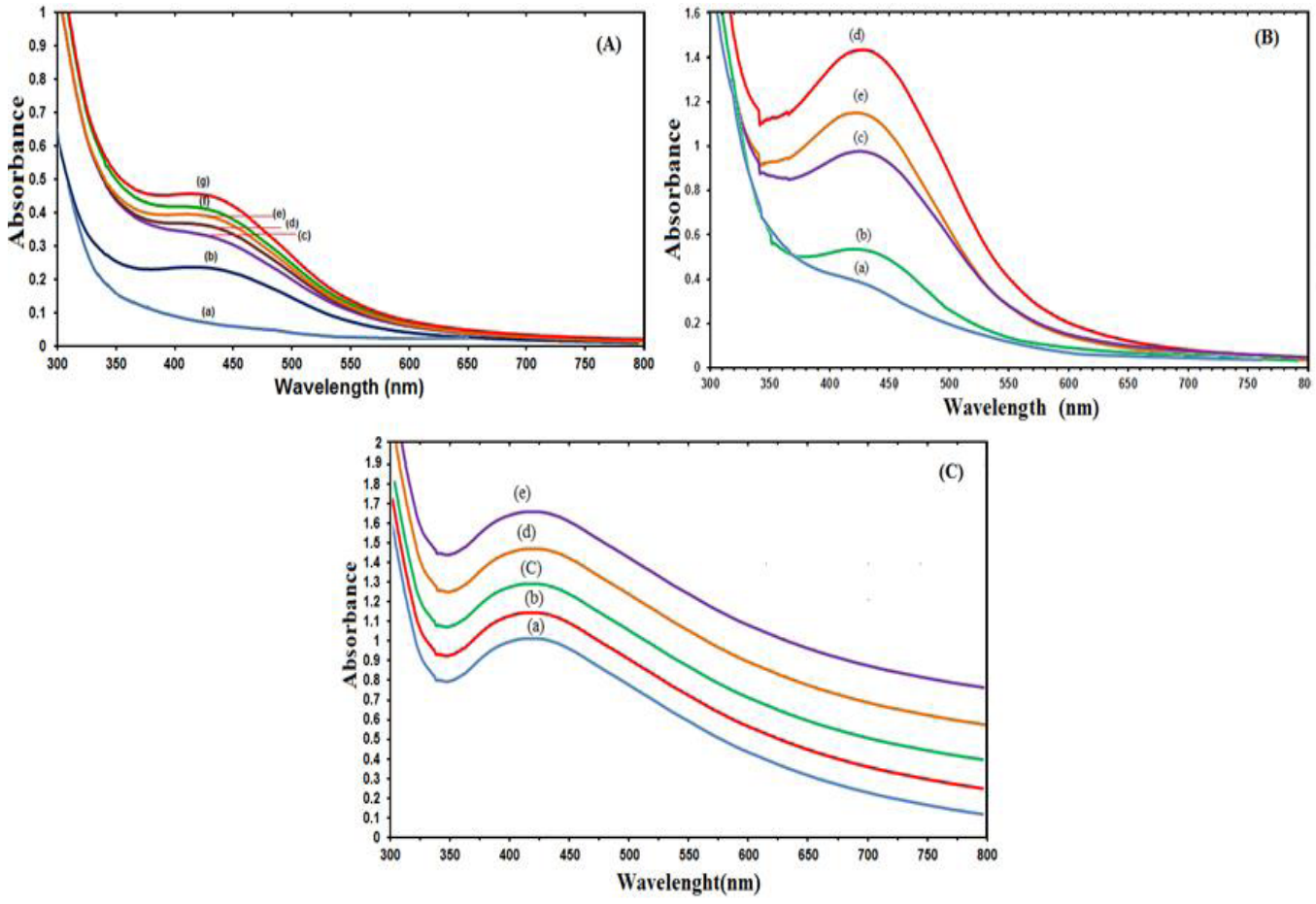

Figure 2: UV–vis spectra of Ag-NPs synthesized under different conditions; (A) ultrasonic irradiation times, (B) silver concentrations and (C) albumin concentrations

Cytological Study on Ag-NPs Effect

The treatment of *Allium cepa* roots by different Ag-NPs (3nm) concentrations with different exposure times caused a significant decrease in mitotic index (Table1). The effect of Ag NPs concentration on mitotic index was significantly different (P<0.05) for 0.0375 and 0.15 M as compared to the control at all times. The lowest value of the MI ***6.37*** was found at 0.15 M concentration after 24 h treatment time. The highest value 21.14 was found at the control of 3 h treatment time. Similar results were reported in *Vicia faba* by Abdel-Azeem and Elsayed (2013) [26]. The obtained data recorded cytotoxic effects after treatment with Ag NPs of 20 nm for 24 hrs as compared to the control.

T. T. (h)	Conc. (M)	ΣCs	XMI ± SD	%ΣADCs	Kinds of Aberrations %						
					St	C. M.	MN	Lag.	F.	Bd.	Dist. A.
3	0	1533	**21.14** ± 0.27	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.0375	1475	20.46 ± 0.09	1.79 ± 0.84	14.29	64.29	0.00	7.14	0.00	14.29	0.00
	0.15	1766	10.57 ± 0.66	2.17 ± 0.29	73.68	0.00	0.00	0.00	0.00	26.32	0.00
6	0	1523	20.75 ± 0.00	0.39 ± 0.28	0.00	33.33	0.00	0.00	0.00	66.67	0.00
	0.0375	2376	16.04 ± 0.13	**2.77** ± 0.34	38.24	0.00	0.00	0.00	5.88	52.94	2.94
	0.15	2094	12.24 ± 0.63	1.48 ± 0.54	47.06	0.00	0.00	0.00	0.00	52.94	0.00
12	0	1532	20.63 ± 0.09	0.00 ± 0.00	0.00	33.33	0.00	0.00	0.00	66.67	0.00
	0.0375	1861	13.59 ± 0.58	2.33 ± 0.49	29.17	8.33	8.33	0.00	4.17	41.67	8.33
	0.15	1703	12.16 ± 0.43	2.56 ± 0.18	85.00	0.00	0.00	0.00	0.00	15.00	0.00
24	0	1518	20.42 ± 0.23	0.26 ± 0.18	0.00	0.00	0.00	0.00	0.00	100.00	0.00
	0.0375	1594	9.39 ± 0.48	2.43 ± 0.56	72.22	0.00	0.00	0.00	5.56	22.22	0.00
	0.15	2552	6.37 ± 0.73	1.02 ± 0.07	92.31	0.00	0.00	0.00	0.00	7.69	0.00

Note: St. = Sticky; **Bd** = Bridge; **F** = Fragment; **Lag.** = Laggards; **CM** = C-metaphase, **MN** = Micronucleus; **Dist. A.** = Disturbed Anaphase

Table 1: Effect of different concentrations of Ag NPs for 3, 6, 12 and 24 h,treatment time (T.T) on mean mitotic index ($\bar{x}$MI ± Standard deviation; SD) and different types of mitotic aberrations in root tips of Allium cepa for different treatment times used in the present study.

Herein, the different treatments of onion roots by Ag NPs caused a significantly higher percentage of chromosomal aberrations than the control indicating a genotoxic effect. The highest percentage (2.77) was found after 0.0375 M treatment for 12 h. Also, the used concentrations of Ag NPs in the present study induced different kinds (bridge, stickiness, disturbed metaphase (CM), disturbed anaphase, laggards and fragments) inTable1 and Fig.3. This agrees with the findings of Kumari *et al.* (2009) and Abdel-Azeem and Elsayed (2013) [26, 27] mitotic index was decreased and several kinds of mitotic aberrations were induced (chromosome stickiness, bridges, breakages and laggards) in *Allium cepa* and *Vicia faba* when treated with Ag NPs. The decrease in mitotic index is an indication of mitodepression and/or cytotoxicity. The mitodepressive and cytotoxic effects might have been achieved by the change in the protein content and/or DNA synthesis as found by Kim and Bendixen (1987) and Sudhakar *et al.* (2001) [28,29]. Cuylen *et al.* (2016) found that chromosomes of cells lacking ki-67 (highly positively charged chromosome protein coat) showed a severe defect in chromosome separation causing stickiness [30]. Ag NPs in the present study could have a similar effect.

Stickiness, and bridges (mostly sticky bridges) were the most frequent kinds of aberrations (Table1). It is a common sign of toxic influence on the chromosomes and is probably an irreversible process. Stickiness was found to be due to inter- and intra-chromosomal cross links involving both DNA-DNA and DNA-protein [31]. However, Patil and Bhat (1992) suggested that stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of chromatin material [32].

Exposure to Ag NPs for 24 h then washed and placed for 72 h of recovery over distilled water, indicated that the inhibition growth compared with the control (Fig.4 a) was associated with typical errors in cell division and chromosome behavior such as; bridge, stickiness, C-metaphase, disturbed anaphase, laggards and fragments. With regard to the root morphology, the control *Allium cepa*

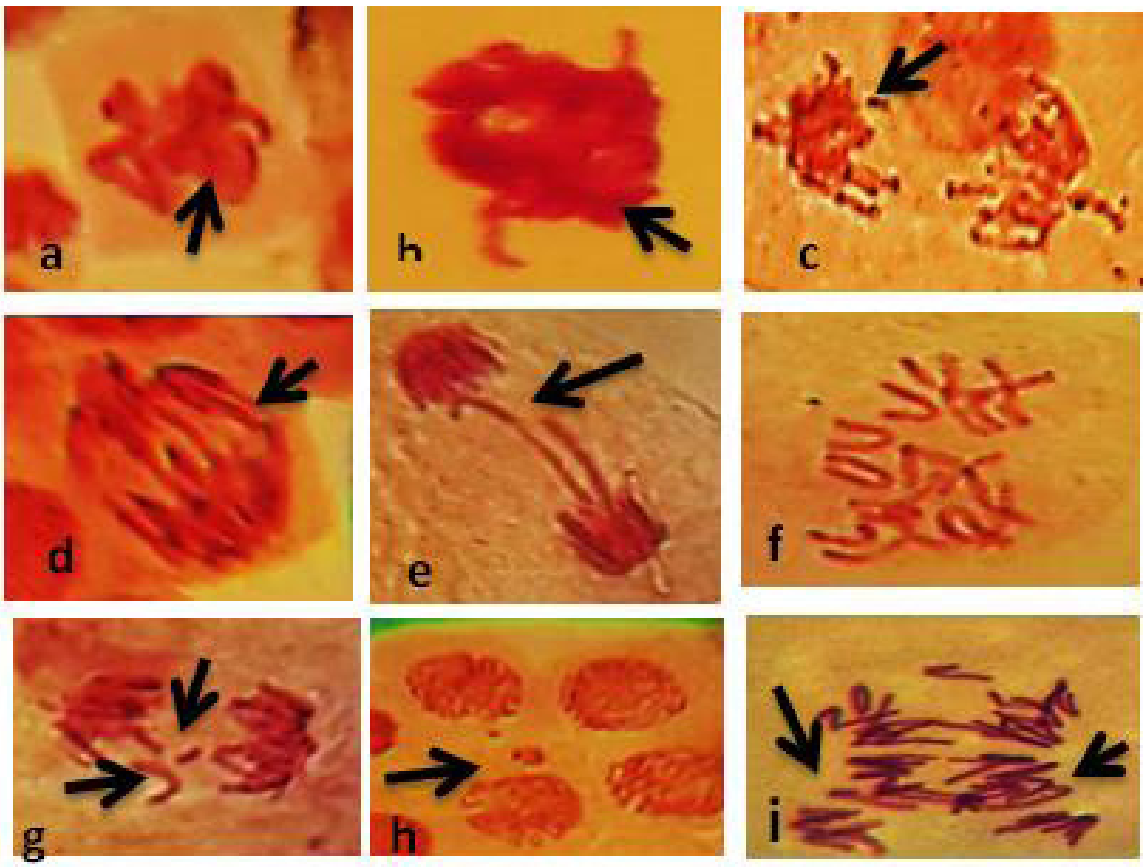

Figure 3: Micrographs showing different abnormal mitotic phases of *Allium cepa* root tip.

a. Sticky metaphase
b. and c. Sticky anaphase
d. and **e.** Multibridge anaphase
f. C-Metaphase
g. Anaphase with a fragment and a lagging
h. Prophase with micronucleus
i. Disturbed anaphase

plants had long roots (5.2 cm), but the plants exposed to AgNPs had much shorter roots (1.6 cm of 0.0375 M and 0.8 cm of 0.15). The brown roots were also observed in both plants of the exposed two concentrations. This indicated that Ag NPs were taken up and accumulated in the plants (Fig.4 b, c). This was concomitant with the findings of Lee *et al.* (2012) cytological analysis on Ag NPs [33]. Additionally Mazumdar (2014) revealed that once Ag NPs enter inside the cells, it may cause damage to the vacuoles and cell wall integrity and probably affect other cell organelles too.

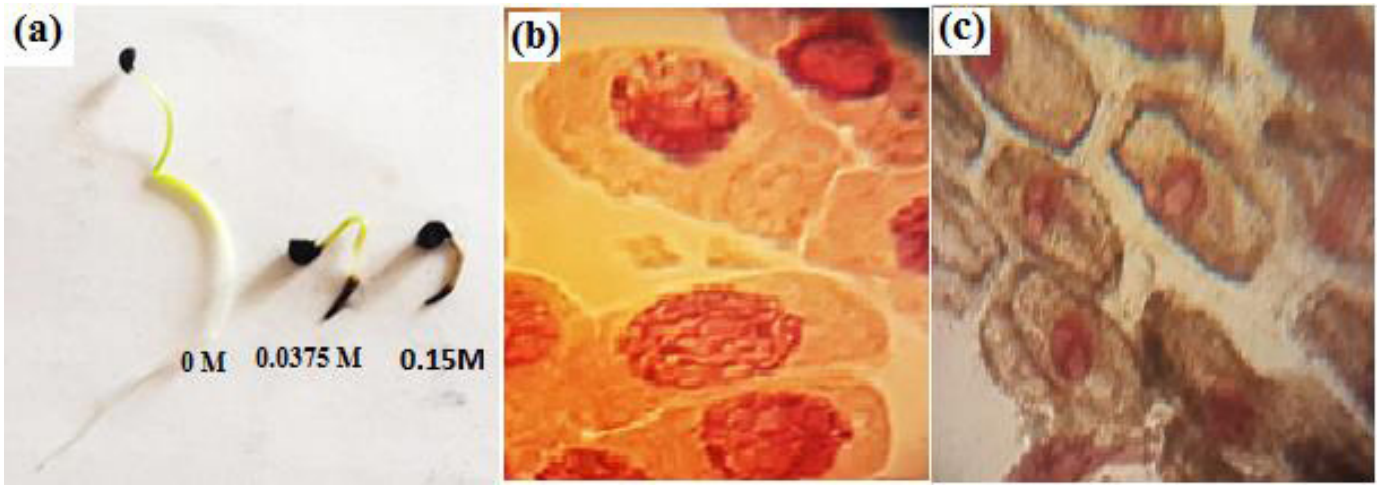

Figure 4: Micrographs showing a. Recovery for 72 h over distilled water. b. Cell examined under microscope at 400X magnification of the control. c. Cell examined under microscope at 400X magnification of the two concentrations of Ag NPs

Conclusion

In sonochemical route, Ag-NPs can be prepared by a green method using $AgNO_3$ as silver precursor and Albumin as a stabilizing. Ultrasonic irradiation time, and concentration of metallic source and the stabilizing agent are the main effective factors in size and yield of Ag-NPs. As the ultrasonic irradiation time, Ag-NPs become smaller and their concentrations are enhanced. The influence of AgNPs on cell division and cytotoxicity improved AgNPs the could penetrate plant system and may impair stages of cell division, causing decreased mitotic index (MI) and increase in chromosomal abnormalities in higher treatments of Ag-Nps and at different time periods; bridge, stickiness, disturbed metaphase (CM), disturbed anaphase, laggards and fragments.

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