Light-regulatory effect on the phytosynthesis of silver nanoparticles using aqueous extract of garlic (*Allium sativum*) and onion (*Allium cepa*) bulb

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Green synthesis emerged as an exciting approach in the field of nanotechnology. Biogenesis of nanosilver is simple, eco-friendly, safe and cost-efficacious and therefore gaining impetus nowadays. An effort has been made to understand the possible induced mechanism for the biosynthesis of silver nanoparticles by exposing a mixture of 1 mM $AgNO_3$ with aqueous extracts of garlic (*Allium sativum*) and onion (*Allium cepa*) under light and dark conditions. In the synthesis of silver nanoparticles, components of garlic and onion bulb extract served both as capping and reducing agents, whereas the light acted as catalyst. Green synthesized silver nanoparticles were quantified spectrophotometrically at different time intervals. Scanning electron microscopy confirmed that biosynthesized nanoparticles were polydispersed, spherical in shape and under the size range of 100 nm. Light proved to be an stimulating factor in the green synthesis of silver nanoparticles which may vary from species to species.

Keywords: Garlic bulb, green synthesis, light effect, onion bulb, silver nanoparticles.

NANOTECHNOLOGY is a rapidly advancing science of producing and utilizing nano-sized particles[1]. Nanoparticles are miniaturized building blocks of bulk substances and therefore serve as the backbone of nanotechnology[2]. In general, a nanoparticle is a microscopic particle with at least one dimension less than 100 nm.

Green chemistry route for nanoparticle synthesis is becoming a nascent branch of nanotechnology that has paved the way for better approaches in the medical field[3]. In order to obtain silver nanoparticles (AgNPs), adoption of plant extract has been used as an intelligible substitute for chemical synthetic protocols[4]. Jose-Yacaman *et al.*[5,6] reported the plant-mediated synthesis of gold and silver nanoparticles. A number of plant varieties such as *Syzygium cumini*[7], *Cadaba indica*[8], *Citrullus colocynthis*[9], *Sesamum laciniatum*[10], *Crocus sativus*[11] and many more[12–17] were reported to synthesize silver nanoparticles. These nanoparticles show completely new properties based on some special attributes such as, size, shape, morphology, distribution, etc.

It is well known that plants have alkaloids, flavonoids and polyphenolic compounds, which can reduce the silver ions to silver nanoparticles and act both as capping as well as stabilizing agents[5]. *Allium* is characterized by the presence of remarkable sulphur-containing compounds, which are responsible for its distinctive smell and pungency. These are considered to possess antibacterial, hypoglycemic and certain other medicinal properties. Several workers[18–22] reported the production of silver nanoparticle using onion extract while a few others[23,24] demonstrated the synthesis of highly stable silver nanoparticles using garlic extract. Various physical and chemical parameters are crucially accountable for the reduction of silver ions. Green route to prepare silver nanoparticles (Ag-NPs) depends on three important parameters, such as solvent medium, reducing or stabilizing or capping agent for Ag-NPs[25,26] and the environment of incubation. Sunlight irradiation using synthetic capping agents[27] and natural capping agents[23] for synthesis of metallic nanoparticles has been reported previously. In view of this, an experiment was designed to explore the effect of light on the potential of garlic and onion bulb extract for rapid phytosynthesis of AgNPs.

Garlic and onion were obtained from the experimental field, Department of Genetics and Plant Breeding, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. Silver nitrate ($AgNO_3$) analytical grade was procured from Sigma Aldrich, USA. All the aqueous solutions were prepared using deionized water.

Accurate concentration of 1 mM silver nitrate was prepared by dissolving, 0.017 g of $AgNO_3$ in 100 ml of deionized water and stored in amber coloured bottle in cool and dry place.

Bulbs of garlic and onion weighing 25 g each were washed thoroughly with deionized water, cut into fine pieces, boiled in 100 ml of deionized water for 10 min, crushed and then filtered with Whatman No. 1 filter paper. Filtrate was stored at 4°C for further use.

For the bioreduction of silver ions, 10 ml of freshly prepared extract and 20 ml of 1 mM silver nitrate solution was added into 170 ml of deionized water with a total reaction volume of 200 ml. The prepared solutions were placed in a dark room (absolute dark) and light conditions (1000 lux) at room temperature in order to provide different light conditions. Appearance of yellowish brown colour was considered as an indicator for green synthesis of AgNps. These colour intensities were recorded using UV-Vis spectrophotometer at different time intervals between 350 and 550 nm.

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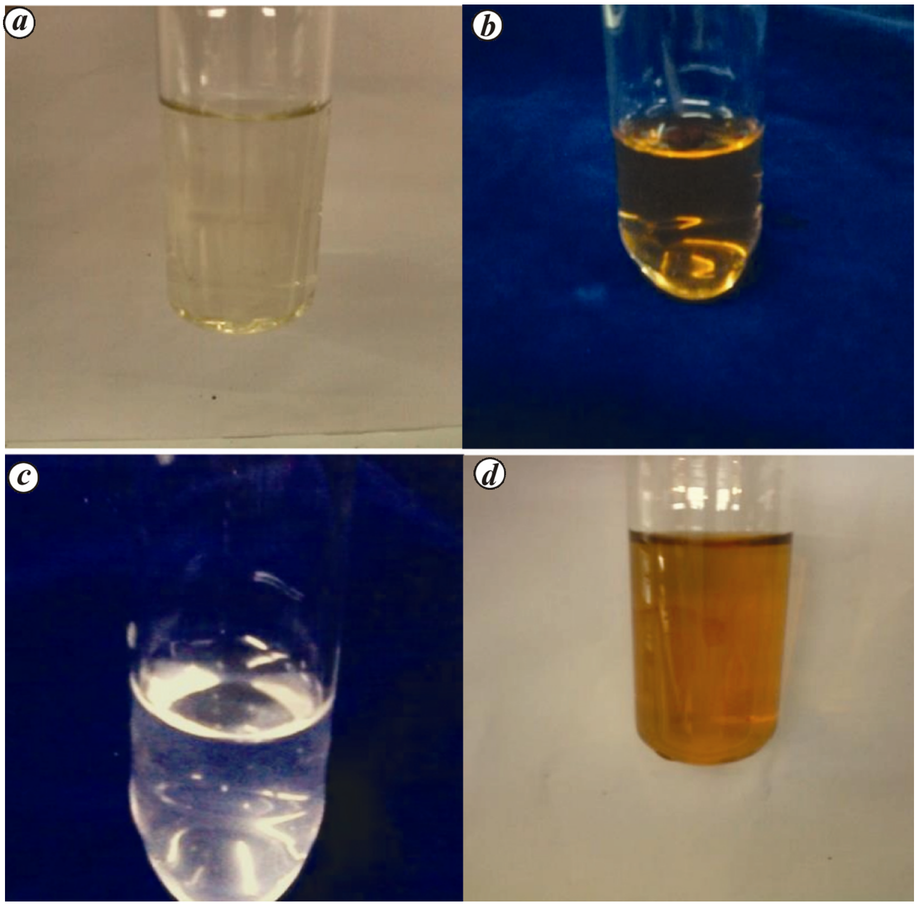

Figure 1. Extract of garlic bulb before (***a***) and after (***b***) addition of 1 mM silver nitrate; Extract of onion bulb before (***c***) and after (***d***) addition of 1 mM silver nitrate, incubated in light conditions.

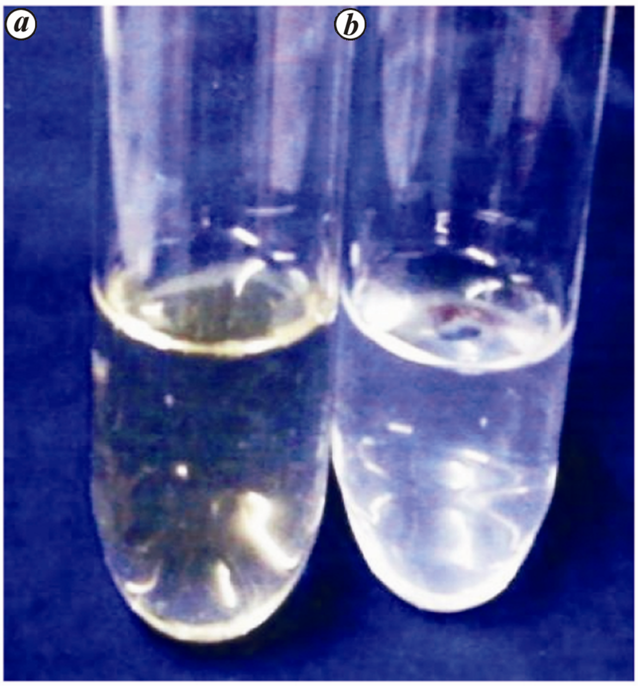

Figure 2. Extract of onion bulb (***a***) and garlic bulb (***b***) after addition of 1 mM silver nitrate incubated in dark conditions.

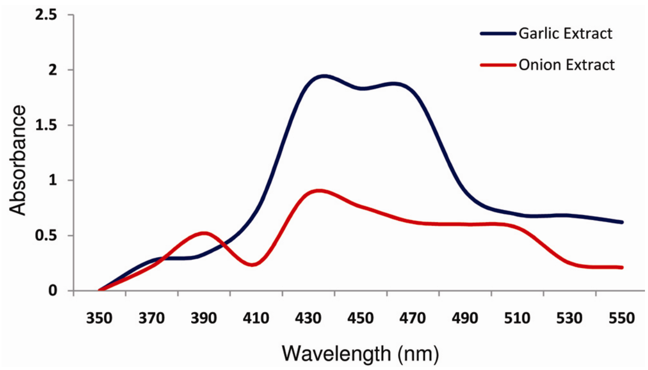

Figure 3. UV-visible spectrum of silver nanoparticles synthesized by garlic and onion extracts exposed to light conditions.

The biosynthesized silver nanoparticles were quantified and characterized through UV-Vis spectra analysis and scanning electron microscopy.

Fabrication of silver nanoparticles by reduction of aqueous silver ions during exposure of garlic and onion was easily determined by UV-Vis spectrophotometer (Model: Systronics double beam spectrophotometer 2202) at 0, 0.5, 1, 6, 12, 18, 24 … 72 h of incubation between wavelength range of 350 and 550 nm.

Table 1. Optical densities of silver nanoparticles synthesized by garlic and onion extracts under light and dark conditions

	Optical density			
	Light conditions		Dark conditions	
Wavelength	Garlic extract	Onion extract	Garlic extract	Onion extract
350	0.00	0.00	–0.06	–0.31
370	0.27	0.22	–0.11	–0.15
390	0.33	0.52	–0.04	–0.08
410	0.72	0.24	0.00	0.00
430	1.87	0.88	0.15	0.40
450	1.83	0.76	0.11	0.24
470	1.80	0.62	0.18	0.17
490	0.90	0.60	0.09	0.10
510	0.69	0.57	0.05	0.08
530	0.68	0.25	0.04	0.05
550	0.62	0.21	0.01	0.02

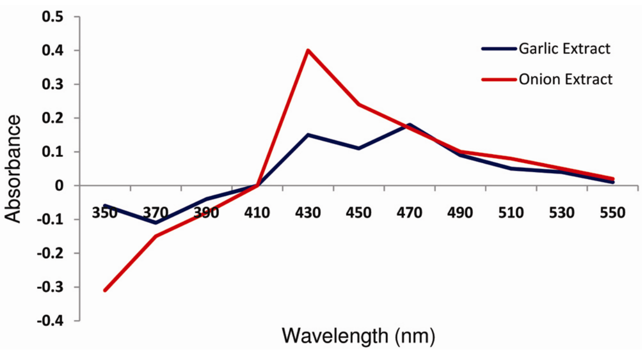

Figure 4. UV-visible spectrum of silver nanoparticles synthesized by garlic and onion extracts exposed to dark conditions.

The size and shape morphology of synthesized silver nanoparticles was recorded by using a scanning electron microscope (Model: JEOL SEI, Japan). Thin film of each sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Garlic and onion are medicinally important plants and are used in many home remedies, as they have anti-oxidant, antibacterial, antihypoglycemic compounds. Garlic possesses allicin[28], an important antihypoglycemic compound, containing s-allyl cysteine sulphoxide a key antioxidant[29], while onion possesses s-methyl cysteine sulphoxide[30], a potent antihypoglycemic compound. These active components are more likely to be responsible for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0).

The aqueous extracts of garlic and onion bulbs were used to produce nanosilver and the appearance of yellowish brown colour in these nanosolutions indicate the synthesis in the light but in the dark condition there is no such indication (Figures 1 and 2). It is illustrious that due to an incitement of surface plasmon oscillations, silver nanoparticles display yellowish brown colour[31] and almost all nanosilver solutions show colour change after incubation.

The nanosolutions of garlic and onion incubated under light intensity of 1000 lux showed the highest absorbance peak at 430 nm. This light-dependent biosynthesis of silver nanoparticles was noticed after 10 min of incubation and continued for 72 h and further showed a stability at 24 h of incubation; the maximum synthesis was observed after 12 h. Under dark conditions, synthesis of silver nanoparticles was delayed and began after 12 h of incubation and continued to 72 h. Results confirmed synthesis of silver nanoparticles under both light and dark conditions. However, synthesis under light was more prominent than dark condition. Reduction of silver ions was observed more in onion extract than garlic extract.

The absorption spectrum of reaction medium confirmed the presence of silver nanoparticles. It uses light in the visible and adjacent (near UV and near-infrared (NIR)) ranges where molecules may undergo electronic transitions[27].

Garlic bulb possesses high efficacy for synthesis of silver nanoparticles under both light and dark conditions. It is more efficient because, it showed swift and utmost synthesis (Figures 3 and 4). Table 1 shows the optical densities of garlic and onion synthesized silver nanoparticles in light condition. Under dark condition, negative values were recorded in both the extracts after addition of $AgNO_3$ at 350–390 nm range, followed by gradual increase in optical density (OD). All the values are original and experiment was performed thrice with five replicates each to confirm reproducibility of results. It could be hypothesized that silver nanoparticles would be emitting some light (through fluorescence and phosphorescence) while interacting with UV light under dark grown conditions. This proposed mechanism of emission of light (through fluorescence and phosphorescence) may be the possible reason for extra transmission of light pertaining to negative values in absorbance. Light plays one of the key influencing factors in chemical reactions. This study concludes that the optimum light is suitable for the biogenesis of silver nanoparticle.

The SEM analysis of green synthesized silver nanoparticles shows that the particles are polydispersed, spheroidal with intermediate size of 100 nm, whereas large nanoparticles showed aggregation (Figure 5). The aggregation may occur because of the cell components present on the surface of silver nanoparticles and can act as capping agents. Nanoparticle size is also reliant on light exposure, i.e. high light absorption will show greater reduction and therefore will synthesize smaller nanoparticles, whereas lesser light absorption will cause lower reduction and therefore lead to production of larger size nanoparticles.

It has been observed that light is significantly executing the green synthesis of silver nanoparticles using onion and garlic extract but this effect is species-specific. Effect of light was more prominent with garlic extract for silver nanoparticle synthesis as compared to onion extract. Under light condition, nanoparticle synthesis started just after mixing of $AgNO_3$ solution in both the extracts, but in dark condition, nanoparticle synthesis was delayed and started only after 12 h and 18 h of incubation in onion and garlic extracts respectively. Phytosynthesis of AgNps is harmonized with photosynthesis as both the processes need light. It can be hypothesized that the production of AgNps is light dependent and it acts as catalyst

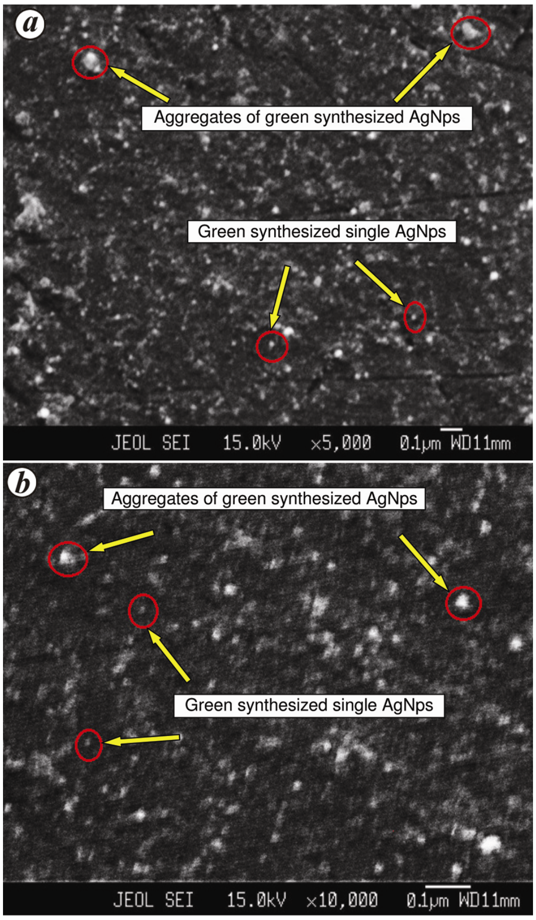

Figure 5. SEM images of synthesized silver nanoparticles. ***a***, Garlic; ***b***, Onion.

for the green synthetic reaction. To elucidate precise mechanism and to comprehend the entire procedure of light-dependent green synthesis of silver nanoparticles, further study is required. The use of biological entities such as plants for the production of nanoparticles yields many ecofriendly and compatible applications for pharmaceutical and biomedical industries. Further betterment in green synthesis, purification and sterilization methods will take nanotechnology to new eco-friendly approach in near future.

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Ameliorative effects of the homeopathic medicine Lycopodium 200c and extract of *Phyllanthus emblica* in cadmium-induced neurotoxicity in mice

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Cadmium is an extremely toxic heavy metal and causes neurotoxicity by inducing oxidative stress and membrane disturbances in brain. *Phyllanthus emblica* and Lycopodium 200c have anti-oxidative properties and are able to remove the cadmium-produced free radicals. This study investigates the role of Lycopodium 200c and *Phyllanthus emblica* (amlaki) in ameliorating the toxic effects of cadmium on the brain of mice. Swiss albino mice were used and divided into four different sets with one control, one induced, one with amlaki and other with both amlaki and Lycopodium treatment. To observe the changes, tests for brain acetylcholinesterase along with Mg^{2+} ATPase activities were performed. Results show that cadmium toxicity leads to decrease in enzymatic activities which can be reversed by the effects of amlaki and Lycopodium 200c.

Keywords: Antioxidative properties, cadmium, free radicals, oxidative stress, toxicity.

AMONG the well-known toxic heavy metals, cadmium is very common which can found in the environment in various compounds, e.g. oxides, sulphides, chlorides, etc. It enters the animal body through food and drinks and is transported through blood and gets easily accumulated in different organs including liver, kidney, testes, lung, etc. and causes severe toxicity. It also acts as a harmful neurotoxin in mammalian brain[1,2]. Cd-induced toxicity is responsible for the generation of reactive oxygen species (ROS)[3,4]. Cadmium also influences the activity of certain enzymes such as the uptake of catecholamines[5] affecting the levels of several neurotransmitters and also affects antioxidant status[6]. It blocks adrenergic and cholinergic synaptic transmissions[7]. There are many chelating agents which form a chelator–metal complex resulting in a decrease of tissue cadmium concentration. Acetylcholinesterase (AChE) is important in the Ach (acetylcholine) cycle[8]. Besides, it is co-released from the dopaminergic neurons[9]. Studies have been conducted to

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