Volume 64, Issue 3, 2020

Journal of Scientific Research

Institute of Science,

Banaras Hindu University, Varanasi, India.

National Conference on Frontiers in Biotechnology & Bioengineering (NCFBB 2020), JNTU Hyderabad, India

Effect of Biosynthesized Silver Oxide Nanoparticles in Increasing the Shelf Life of Different Foods

Sunita. D[*1], Jullian Yegoti[1]

[1]Department of Microbiology, St Francis College for Women, Begumpet, Hyderabad, Telangana.

sunitagnk@gmail.com

Abstract: **The Silver oxide nanoparticles have numerous significant applications and they are well-known for disinfecting effect. They are used in traditional medicines and to synthesize various products. It is been identified that Silver oxide nanoparticles are not toxic to humans and are active against microbes at low concentrations and they have no side effects. Furthermore, numerous salts of silver and its derivatives are manufactured as antimicrobial agents. Small concentrations of silver is safe for human cells, yet toxic for microorganisms. To prepare these Silver oxide nanoparticles, although chemical and physical methods are existing for making well defined nanoparticles, these methods are pretty expensive and potentially harmful to the environment. Use of normal microorganisms, plant extract and plant biomass may perhaps be alternative to chemical and physical methods for production of nanoparticles in an ecological way. Various biowaste materials and microorganisms can be used for synthesis of Silver oxide nanoparticles and their antibacterial activity can be assessed on organisms causing spoilage of different vegetables and fruits.**

The synthesis of Silver oxide nanoparticles in a biological way materializes as an eco-friendly and stimulating approach in the area of nanotechnology. In the present study, apple, pomegranate, orange, cucumber, potato, watermelon peel extracts and Probiotic *E. Coli* were utilized for synthesis of Silver oxide nanoparticles and their antibacterial activity was calculated. The peel extracts were used as capping agents to reduce silver nitrate solution to Silver oxide nanoparticles. Later, these nanoparticles were characterized and coated onto fresh fruits and vegetables to check whether their shelf life has been increased.

Keywords: **Silver oxide Nanoparticles, Biosynthesis, *E. Coli*, Spectrophotometry, Antimicrobial activity.**

I. INTRODUCTION

Nanotechnology is currently generating an increasing sense of interest in the life sciences particularly in biomedical devices and biotechnology (Krishnananda P ingle et al., 2017). Nanoparticles of some metals like Gold, Silver oxide, Titanium, Iron, Zinc, Carbon, Copper, Palladium and Platinum are broadly used in making of products such as shampoos, soaps, detergents, shoes, cosmetic products, and tooth paste, besides medical and pharmaceutical applications (Ritu Gupta and Huan Xie, 2018). Several nanomaterials are employed as antimicrobial agents in food packing in which silver oxide nanoparticles are of abundant interest. This is because of its extended use (Marilena Carbone et al., 2016).

The other nanoparticles currently used are titanium dioxide, zinc oxide, silicon oxide, magnesium oxide and gold. Though chemical and physical approaches make successful and pure, well defined nanoparticles, these methods are pretty expensive and potentially harmful to the environment (Jagpreet Singh et al., 2018). Use of natural microorganisms, plant extract and plant biomass may perhaps be substitute to chemical and physical methods for manufacture of nanoparticles in an ecological manner (Coreena Fernandez et al., 2016).

The silver oxide nanoparticles have numerous important applications and are known to own a disinfecting effect and have applications extending from traditional medicines to various products (S. P. Deshmukh et al., 2019). It is been identified that silver oxide nanoparticles are not toxic to humans and are effective against microbes at low concentrations and they have no side effects. Furthermore, numerous salts of silver and its derivatives are manufactured as antimicrobial agents. Small concentrations of silver are safe for human cells, yet toxic for microorganisms (Thirugnanasambandan et al., 2011). Bio Waste materials and microorganisms may be used for synthesis of silver oxide nanoparticles. The antibacterial activity can be evaluated on organisms causing spoilage of various foods (Simbine et al., 2019).

Silver oxide nanoparticles are known to have inhibitory and bactericidal effects (Mehdi Zarei et al., 2014). In the present study, apple, pomegranate, watermelon, orange, cucumber and potato peel extracts were used for synthesis of silver oxide nanoparticles. Their antibacterial activity was evaluated. In the current study the synthesis of silver oxide nanoparticles is through different fruit peels, vegetable peels and probiotic *E.*

[*] Corresponding Author

DOI: 10.37398/JSR.2020.640302

Selection and Peer-review under responsibility of the Program Chairs.

coli. The naturally synthesized silver oxide nanoparticles have a prominent role in preventing spoilage of fresh foods owing to their anti microbial nature. The Antimicrobial effect of the synthesized silver oxide nanoparticles was studied by performing agar well diffusion method. The most important study made was coating of silver oxide nanoparticles onto fresh vegetables and fruits and observations made regarding whether their shelf life had been increased.

II. MATERIALS AND METHODS

A. Isolation of bacteria from spoiled vegetables and fruits:

- Spoiled vegetables and fruits are collected from local markets.
- The spoiled vegetable and fruit samples have been serially diluted and inoculated on to the nutrient agar plates and incubated at 37^0C for 24hrs (N. A. Hasanand I. M. Zulkahar, 2018).

B. Characterization of bacteria from spoiled vegetables and fruits:

- The isolated colonies are taken for further steps of characterization by Bergey's Manual.
- The bacteria isolated from the nutrient agar plates were identified based on its Gram nature, morphological characters and biochemical tests (Lalita Chaudhary and T. S. Dhaka, 2016).

C. Microbial synthesis of silver oxide nanoparticles:

- Nuritent broth was prepared.
- 0.1ml *E.coli* culture was inoculated into 50ml of broth and incubated at 37^oC for 3days.
- The broth was centrifuged at 10,000 rpm for 10mins.
- 15ml of *E.coli* supernatant was treated with 40ml of 1mM $AgNO_3$ solution.
- Change in colour of the sample was observed which showed a brown colour.
- Absorbance values to be taken in the range of 400-530nm using UV-VIS Spectrophotometer (Vibha Saklani et al., 2012; H.R. Ghorbani, 2013).

D. Bio synthesis of silver oxide nanoparticles from vegetable and fruit peels:

- Apple, Pomegranate, Water melon, Orange, potato, cucumber peels were collected and cleaned thoroughly using distilled water.
- The fresh vegetables &fruits were then kept under sunlight for complete drying, for 3 to 4 days.
- After drying, the peels were cut into small pieces and grinded into powder form.
- 25g of the fruit peel powder was added to 50 ml of distilled water and boiled for 10 min at 80°C.
- The peel extracts were filtered using Whatman's filter paper and the filtrates were stored in conical flasks at 4°C for further use.
- In typical biosynthesis process, 1ml of the peel extract was added to 9ml of double distilled water and solution made up to 10ml in a test tube.
- The synthesis of silver oxide nanoparticles involves addition of 1mM $AgNO_3$ solution to 10ml of filtrate in test tubes and kept under dark conditions for 48 hours. Peel extracts, without addition of silver nitrate were kept as control (Anil R Shet, 2016; Ruchi Patel and Dr. Mehali Mehta, 2019; Sanket Kaushik and Anupam Jyoti, 2016).

E. Characterization of silver oxide nanoparticles:

- Characterization of Silver oxide Nanoparticles was done by UV-VIS Spectrophotometry. Absorbance values were taken between 300nm to 500nm. Silver oxide nanoparticles produce a peak around 420nm (Hamouda. R.A et al., 2019).

F. Study of antimicrobial activity of silver oxide nanoparticles:

- Study of Antimicrobial activity of Silver oxide nanoparticles was done by Agar well diffusion method and various aliquots of silver oxide nanoparticle solutions (Loo Y. Y et al., 2018).

G. Coating of silver oxide nanoparticles onto fruits & vegetables:

- The Silver oxide nanoparticles which are synthesized; are coated onto the fruits and vegetables and kept under observation at room temperature for 7 days to check for delay of spoilage (P. Balashanmugam et al., 2014).

III. RESULTS

A. Isolation and characterization of bacteria from spoiled fruits & vegetables

Table –1: Bacteria Isolated From Spoiled Vegetables:

Spoilt vegetable	Tomato	Cucumber	Brinjal	Lady's finger	Capsicum
Bacteria identified	*Pseudomonas sps* *Enterobacteriaceae*	*Pseudomonas sps* *Staphylococcus sps*	*Pseudomonas sps* *Staphylococcus sps*	*Staphylococcus sps*	*Staphylococcus sps*

Table - 2: Bacteria isolated from spoiled fruits:

Spoilt Fruit	Apple	Orange	Water melon	Banana
Bacteria identified	*Corynebacterium kutsceri* *Clostridium spp*	*Clostridium spp*	*Clostridium spp*	*Corynebacterium kutsceri* *Clostridium spp* *Staphylococcus sps*

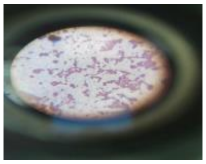
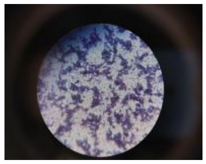



Fig 1: Gram -ve Bacilli Fig 2: Gram +vecocci


Fig 3: Catalase test Fig 4: Oxidase test

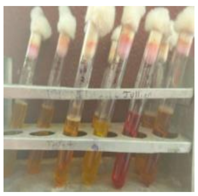
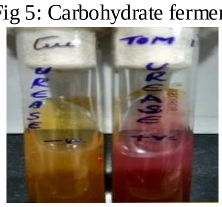



Fig 5: Carbohydrate fermentation test Fig 6: Indole test



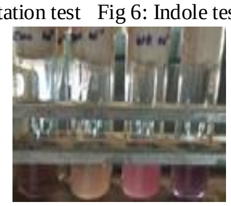


Fig 7: Urease test Fig 8: Ornithine decarboxylase test

B. Preparation of Silver oxide nanoparticles from peels & bacteria:

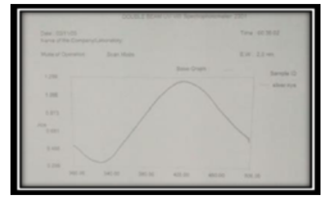
Fig 9: Crude Extract Powders of Peels

Fig 10: Silver oxide Nps Prepared Using *E.coli*

Fig 11: Silver oxideNps solutions

C. Characterizationof silver oxide nanoparticles using UV - VIS spectrophotometry:

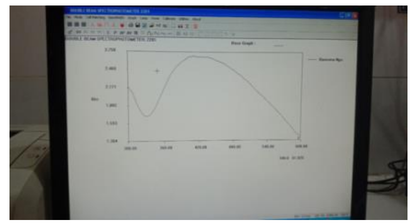
Y-Axis - Absorbance



X- Axis – Wave length in mm

Fig 12: Silver oxide Nps from *E. coli*

Y- Axis - Absorbance



X- Axis – Wave length in mm

Fig 13: Silver oxide Nps from Apple peels

D. Antimicrobial Activity of Silver oxide Nanoparticles:

Table - 3: Antibacterial activity of Silver oxide Nps Synthesized Using *E.coli*

Bacteria	***E. coli*Nps : Zone of inhibition(cm)**				
	50 µl	80 µl	110 µl	140 µl	170 µl
Staphylococcus sps	2.1	2.3	2.5	2.9	3.9
Pseudomonas sps	2.1	2.1	2.2	2.2	2.6
Enterobacteriaceae	2.4	2.4	2.5	2.5	2.5

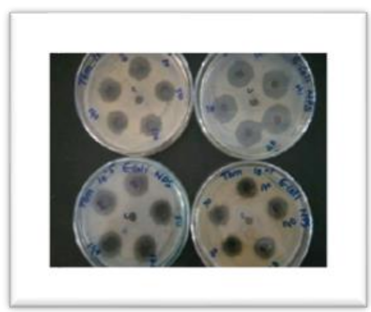


Fig 14: Antibacterial activity of Silver oxide Nps Synthesized Using *E.Coli*

Table - 4: Antibacterial Activity of Silver oxide Nps Synthesized from Apple Peel

Bacteria	**Apple Nps : Zone of inhibition (cm)**				
	50 µl	80 µl	110 µl	140 µl	170 µl
Staphylococcus sps	1.9	2.0	2.4	2.6	2.8
Pseudomonas sps	1.8	1.9	1.9	2.0	2.2
Enterobacteriaceae	1.6	1.7	1.7	1.9	1.9

Fig 15: Antibacterial Activity Of Silver oxide Nps Synthesized from Apple Peel

Table- 5: Antibacterial Activity of Silver oxide Nps Synthesized from Pomegranate Peel

Bacteria	Pomegranate Nps : Zone of inhibition (cm)				
	50µl	80µl	110µl	140µl	170 µl
Staphylococcus sps	1.2	1.3	1.4	1.5	1.6
Pseudomonas sps	1.0	1.2	1.3	1.4	1.5
Enterobacteriaceae	1.5	1.6	1.9	1.9	2.0

Fig 16: Antibacterial Activity of Silver oxide Nps Synthesized from Pomegranate Peel

Table-6: Antibacterial Activity of Silver oxide Nps Synthesized from Cucumber Peel

Bacteria	Cucumber Nps: Zone of inhibition (cm)				
	50 µl	80 µl	110 µl	140 µl	170 µl
Pseudomonas sps	1.6	1.8	1.9	1.9	2.0
Staphylococcus sps	1.2	1.3	1.3	1.4	1.6
Enterobacteriaceae	-	-	1.4	1.9	2.0

Fig 17: Antibacterial Activity of Silver oxide Nps Synthesized from Cucumber Peel

Table-7: Antibacterial Activity of Silver oxide Nps Synthesized from Orange Peel

Bacteria	Orange Nps : Zone of Inhibition (cm)				
	50 µl	80 µl	110 µl	140 µl	170 µl
Pseudomonas sps	1.8	1.8	2.5	2.6	3.0
Staphylococcus sps	1.5	1.7	1.8	1.9	2.0
Enterobacteriaceae	1.5	1.5	1.6	1.6	1.7

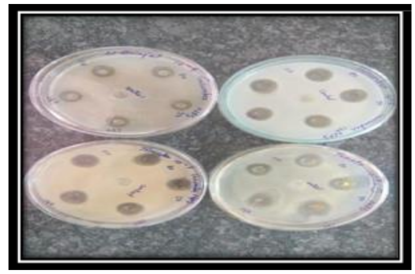


Fig 18: Antibacterial Activity of Silver oxide Nps Synthesized from Orange Peel

Table-8: Antibacterial Activity of Silver oxide Nps Synthesized from Potato Peel

Bacteria	Potato Nps : Zone of Inhibition in cm				
	50 µl	80 µl	110 µl	140 µl	170 µl
Pseudomonas sps	1.9	2.0	2.2	2.3	2.4
Staphylococcus sps	1.0	1.1	1.4	1.6	1.7
Enterobacteriaceae	1.6	1.8	1.9	2.0	2.0

Fig.19. Antibacterial Activity of Silver oxide Nps Synthesized from Potato Peel

Table- 9: Antibacterial Activity of Silver oxide Nps Synthesized from Watermelon Peel

Bacteria	Water melon Nps : Zone of Inhibition (cm)				
	50µl	80 µl	110 µl	140 µl	170 µl
Pseudomonas sps	1.3	1.4	1.5	1.7	1.9
Staphylococcus sps	2.3	2.3	2.5	3.0	3.5
Enterobacteriaceae	1.5	2.0	2.4	2.8	3.0

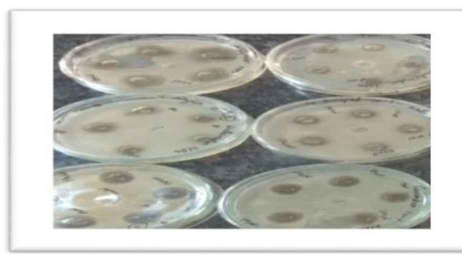


Fig 20. Antibacterial Activity of Silver oxide Nps Synthesized from Watermelon Peel

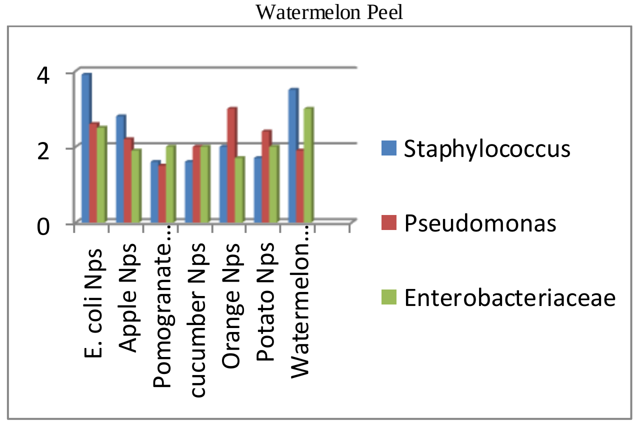


Fig 21. Comparative analysis of Antibacterial activity of various silver oxide Nps against bacteria

E. Silver oxide nanoparticles coated onto vegetables and fruits

Fig 22: Fruits coated with Silver oxide Nps

Fig 23. Vegetables coated with Silver oxide Nps

IV. DISCUSSION

Bearing in mind the present demands of the world, this work had focused on the synthesis of different silver oxide nanoparticles from bio wastes and applications of them in our daily life will completely revolutionize the modern era. Several types of bacteria were isolated from spoiled fruits and vegetables by plating method. The isolated bacteria were characterized following Bergey's manual and found to be *Pseudomonas sps*(fig 4) , *Staphylococcus sps* (fig 2, 3), *Enterobacteriaceae* (fig 1, fig 5, fig 6, fig 7, fig 8), *Corynebacterium kutscheri* (fig 3) and *Clostridium sps*. Microbial synthesis of silver oxide nanoparticles was done by using probiotic strain of *E.Coli* and silver nitrate solution (fig 10). In the same way nanoparticles were synthesized from the dried fruit and vegetable peel extracts of apple, pomegranate, cucumber, orange, potato and watermelon, in combination with the silver nitrate solution (fig 11). The synthesized silver oxide nanoparticles gave peak around 420nm, when characterized in UV – VIS spectrophotometry confirming the existence of silver oxide nanoparticles (Karunagaran Vithiya & Rajendran Kumar, 2014) (fig 12, fig 13).

Antibacterial activity of the silver oxide nanoparticles were checked against the isolated bacteria by performing agar well diffusion method. Antibacterial activity of the synthesized silver oxide nanoparticles was found against the isolated spoilage causing bacteria. Zones of inhibition were measured. *Staphylococcus sps*, *Pseudomanas sps* and *Enterobacteriaceae* organisms shown inhibition in varied range (Mehdi Zarei et al., 2014). Among all the types of silver oxide nanoparticles, the ones prepared using *E. coli* showed maximum antibacterial activity against *Staphylococcus sps* with a zone of diameter value 3.9cm (table 3, fig 21). After performing the antibacterial activity, it was found that the Silver oxide nanoparticles which were synthesized using the probiotic strain of *E.coli* were the most efficient as they showed a higher zone of inhibition around the microbial colonies, followed by Silver oxide nanoparticles synthesized from various peels. As these Nanoparticles were synthesized using non pathogenic strain of *E.coli*, they can be applied in our everyday life to increase the shelf life of various fruits and vegetables (H.R. Ghorbani 2013).

Silver oxide nanoparticles synthesized using watermelon peels exhibited maximum antibacterial activity against *Staphylococcus sps* with an inhibition zone of 3.5cm and against *Enterobactericeae* with a zone of diameter value 3.0cm (table 9). Silver oxide nanoparticles synthesized using apple peels shown maximum antibacterial activity against *Staphylococcus sps* with zone of 2.8cm (table 4). Silver oxide nanoparticles synthesized using pomegranate, potato & cucumber peels shown maximum antibacterial activity against *Enterobacteriaceae* with zone of 2.0cm (tables 5, 6, 8). Silver oxide nanoparticles synthesized using orange peels shown maximum antibacterial activity against *Pseudomonas sps* with 3.0cm zone & *Staphylococcus sps* with zone of 2.0cm (Kuldeep Sharma et AL., 2016) (table 7). These microbial and biosynthesized silver oxide nanoparticles, when coated onto various fruits and vegetables, have shown to maintain their freshness for more time period when compared with uncoated fruits and vegetables (fig 22, fig 23). The fruits & vegetables coated with silver oxide nanoparticles did not develop spots, shrinkage and were looking fresh compared to uncoated foods (P. Balashanmugam, R. Nandhini et al., 2014).

CONCLUSION

The physicochemical properties of silver oxide nanoparticles with the inhibitory capacity against microbes has increased the research on nanoparticles and their potential application as antimicrobials. The direct coating of silver nanoparticle is reported in this research. Silver nanoparticles can serve as a protection by delaying the ripening of fruit and vegetables. Silver nanoparticles not only has antimicrobial property but also the capability to retard oxygen diffusion and it is resistant to oxidation. The naturally synthesized silver oxide nanoparticles can be experimented and used in a large scale by the farmers to surge the shelf life of fruits and vegetables. This may also bring down the cost of cold storage for the farmers, increasing the shelf life of fruits and vegetables. So, microbial and bio synthesized silver oxide nanoparticles can be employed as an alternative to chemical preservatives on various foods.

ACKNOWLEDGEMENT

I sincerely thank UGC for funding the work. I also thank the management of St Francis college for women and Dr. Shailaja

Raj, Head, Department of Microbiology, St Francis college for women for their constant support in conduct of the work.

REFERENCES

Anil R Shet, Shwetha Tantri & Arvind Bennal (2016). Economical biosynthesis of silver nanoparticles using fruit waste. *Journal of chemical and pharmaceutical sciences*, 9 (4), 2306-2311.

Coreena fernandez, Anitha thomas & M. Shailaja raj (2016). Green synthesis of silver oxide nanoparticle and its antimicrobial activity against organisms causing Dental plaques. *International Journal of Pharma and Bio Sciences*, 7 (4), 14-19.

Hamouda, R.A., Hussein, M.H., Abo-elmagd, R.A. et al., (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatorialimnetica*. *Scientific Reports* 9, 13071.

H.R. Ghorbani (2013). Biosynthesis of Silver Nanoparticles by *Escherichia coli*. *Asian Journal of Chemistry*, 25(3), 1247-1249.

Jagpreet Singh, Tanushree Dutta, Ki-Hyun Kim, Mohit Rawat, Pallabi Samddar & Pawan Kumar (2018). 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of Nanobiotechnology*, 16, 84.

Karunagaran Vithiya & Rajendran Kumar & Sen Shampa (2014). Antimicrobial Activity of Biosynthesized Silver Oxide Nanoparticles. *Journal of Pure and Applied Microbiology*, 4, 3263-3268.

Krishnananda P Ingle, Amit G Deshmukh, Dipika A Padole, Mahendra S Dudhare, Mangesh P Moharil & Khelurkar VC (2017). Silver nanoparticles: A new Era of nanobiotechnology. *The Pharma Innovation Journal*, 6(1), 01-04.

Kuldeep Sharma, Sanket Kaushik, Anupam Jyoti (2016). Green Synthesis of Silver Nanoparticles by Using Waste Vegetable Peel and its Antibacterial Activities. *Journal of Pharmaceutical research Sciences*, 8(5), 313-316.

Lalita Chaudhary & T. S. Dhaka (2016). Isolation and identification of bacteria from some spoiled fruits. *Plant Archives*, 16 (2), 834-838.

Loo YY, Rukayadi Y, Nor-Khaizura M-A-R, Kuan CH, Chieng BW, Nishibuchi M &Radu S (2018). In Vitro Antimicrobial Activity of Green Synthesized Silver Nanoparticles Against Selected Gram-negative Foodborne Pathogens. *Frontiers in Microbiology*, 9, 1555.

Marilena Carbone, Domenica Tommasa Donia, Gianfranco Sabbatella & Riccarda Antiochia (2016).Silver nanoparticles in polymeric matrices for fresh food packaging. Journal of King Saud University - Science, *28 (4), 273-279.*

Mehdi Zarei, Amirhesam Jamnejad & Elahe Khajehali (2014). Antibacterial Effect of Silver Nanoparticles Against Four Foodborne Pathogens. *Jundishapur Journal of Microbiology*, 7(1), e8720.

N.A. Hasan & I. M. Zulkahar (2018). Isolation and identification of bacteria from spoiled fruits. *AIP Conference Proceedings 2020*,020073(2018); https://doi.org/10.1063/1.5062699

P. Balashanmugam, R. Nandhini, V. Vijaya priyadharshini & P.T Kalaichelvan (2014). Biosynthesis of Silver Nanoparticles from Orange Peel Extract and its Antibacterial Activity against Fruit and Vegetable Pathogens. *International Journal of Innovative Research in Science & Engineering*, ISSN (Online) 2347-3207.

Ritu Gupta & Huan Xie (2018).Nanoparticles in Daily Life: Applications, Toxicity and Regulations. *Journal of environmental pathology, toxicology and oncology*, 37(3), 209 – 230.

Ruchi Patel & Dr. Mehali Mehta (2019). Green Synthesis of Silver Nanoparticles by using Fruit and Vegetable Waste: a Review. *Journal of Engineering Research and Application*, 9(3), 78 – 85.

Simbine, Emeldaorlando, Rodrigues, Larissa da cunha, Lapa-guimarães, Judite, Kamimura, Elianasetsuko, Corassin, Carlos humberto, & Oliveira, Carlos augustofernandes de (2019). Application of silver nanoparticles in food packages: a review. *Food Science and Technology*, 39(4), 793-802.

S.P. Deshmukh, S.M. Patil, S.B. Mullani & S.D. Delekar (2019). Silver nanoparticles as an effective disinfectant: A review. Materials Science and Engineering *C: Materials for Biological Applications*, 97, 954–965.

Thirugnanasambandan, Theivasanthi & Alagar, Marimuthu (2011). Anti-bacterial Studies of Silver Nanoparticles. *Mendeley*, 1101.

Vibha Saklani, Suman & V.K. Jain (2012). Microbial Synthesis of Silver Nanoparticles: A Review. *Journal of biotechnology & biomaterials*, S13:007, doi:10.4172/2155-952X.S13-007.

***