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GREEN SYNTHESIS: A NOVEL APPROACH FOR NANOPARTICLES SYNTHESIS

Repudaman Singh Sandhu [1], Ravindra Prasad Aharwal [2] and Suneel Kumar [*3]

Department of Mechanical Engineering [1], Lovely Professional University, Jalandhar - 144411, Punjab, India.
Department of Botany [2], Rajabhoj Government College, Katangi, Balaghat - 481445, Madhya Pradesh, India.
Bio-Design Innovation Centre [3], Rani Durgavati University, Jabalpur - 482001, Madhya Pradesh, India.

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Correspondence to Author:
Dr. Suneel Kumar

Faculty of Bio-Design Innovation Centre, Rani Durgavati University, Saraswati Vihar, Pachpedi, Jabalpur - 482001, Madhya Pradesh, India.

E-mail: sunilsingh07604@gmail.com

ABSTRACT: There are different types of conventional approaches are used for the synthesis of nanoparticles like physical and chemical techniques. But above approaches used for nanoparticles synthesis is not eco-friendly due to the production of toxic compounds during nanoparticles formation. Therefore, interest in the green synthesis of nanoparticles has been increased. The green synthesis of nanoparticles is an eco-friendly and convenient approach in which there are no adverse effects on the environment. The production of nanoparticles by green synthesis minimizes time and can obtain desired size and shape to increase the constancy of nanoparticles. The living organisms like plant, bacteria, algae, and fungi are known to be capable for production of metals nanoparticles through the intracellular or extracellular mode of biosynthesis. The main reason of involvement of plants, algae especially microorganisms in the production of metal nanoparticles are to develop, immaculate, harmless and environmentally protected production processes for nano synthesis to diminish environmental impact, minimize waste and boost energy efficiency. Therefore, the present review focus on methods concerned on the synthesis of nanoparticles, efforts to merge extensive data reported and methods applied for the synthesis of nanoparticles by using various microbes, algae and plant extracts as well as different techniques involved in the characterization of nanoparticles.

INTRODUCTION: The area of nanoparticles synthesis is growing up day by day due to their secure and eco-friendly procedure. The nanoparticles have a significant role in the field of pharmaceutical sciences, biotechnology, farming and therapeutic discipline [1, 2]. The nanotechnology is a combination of Science and Technology has the ability for designing, characterization, production and utilization of nanoparticles.

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By using this approach, we can control the shape and dimension of metal ions at a very small stage. The nanotechnology develops nanoparticles from the matter at the very small stage that show different properties in the area of drug delivery system, diagnostics, nano-drug treatment, biomarkers, cell labeling and also act as antimicrobial and anticancer agents [3-6]. A variety of physical and chemical techniques are used for the synthesis of nanoparticles [7, 8]. But synthesis of metal nanoparticles by physical and chemical procedure released harmful compounds which are injurious to human health and environment. Thus, an alternative approach for the synthesis of metal nanoparticles is a biological method that involves synthesis of nanoparticles by the plant, fungi, algae, bacteria yeast *etc*.

Due to simple, cost-effective and eco-friendly way of nanoparticles synthesis through the biological system is most assure field of nanoparticles production in present time. The nanoparticles synthesized by microorganisms reveal the high surface area to volume ratio, small size, spatial internment, thermal, magnetic, catalytic and biological properties [9, 10, 11, 12]. The biological synthesis of metals nanoparticles gets a huge consideration in the field of nanomedicine and pharmaceutical sciences due to their inimitable properties which can be used in the treatment of different types of ailments. The silver nanoparticles (AgNP) have a vital function in disruption of the mitochondrial respiratory system that is responsible for the generation of reactive oxygen species which induced DNA destruction and increase apoptosis during cancer development in organisms [13, 14]. The silver nanoparticles attached with the cell membrane of bacteria and change their confirmation which leads to cell death and also bind with a thiol group and inactivates the actions of enzymes in bacteria [15, 16, 17, 18]. The silver metal is a soft base which reacts with an acid group like phosphate and sulphar present in DNA that lead to cell death as shown in **Fig. 1**. In most of the cases silver nanoparticles dephosphorylates peptide substrate on tyrosine residue in Gram negative bacteria that produce signal transduction blockade to prevent the growth of bacteria [19].

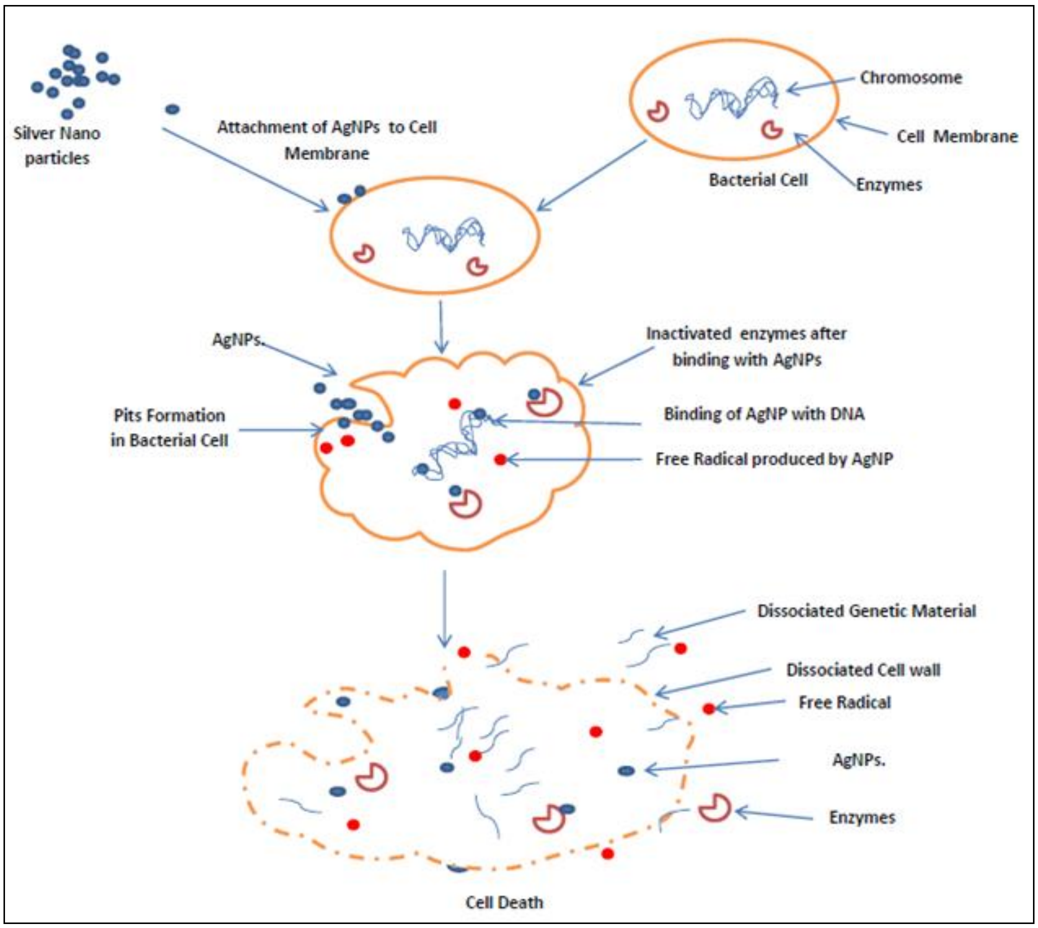

FIG. 1: MECHANISM OF AgNP IN BACTERIAL CELL DEATH

Methods Involve in Synthesis of Nanoparticles: In the present scenario, a variety of physical, chemical and biological methods are used for the synthesis of metal nanoparticles which have wide application in the field of medical, research and pharmaceutical sciences. The physical methods often used in synthesis of nanoparticles are diffusion, irradiation, thermal decomposition, and arc discharge radiation assisted [7], thermal decomposition [8], laser ablation [20], sonochemical [21], photochemical [22], polyaniline synthesis [23], ball milling, electrochemical techniques and chemical methods used as chemical reduction, condensation, sol-gel method precipitation and laser pyrolysis *etc*. **Fig. 2.** But most capable and eco-friendly approach which is more reliable and cost-effective is the biological procedure for the synthesis of nanoparticles through bacteria, fungi, algae, plants, actinomycetes *etc*. The production of consistent and nonhazardous synthesis of nanoparticles can be possible through the biological system. But, it is also important to elaborate this technique of green synthesis of nanoparticles through living organisms at a commercial level for their expected outcomes [24].

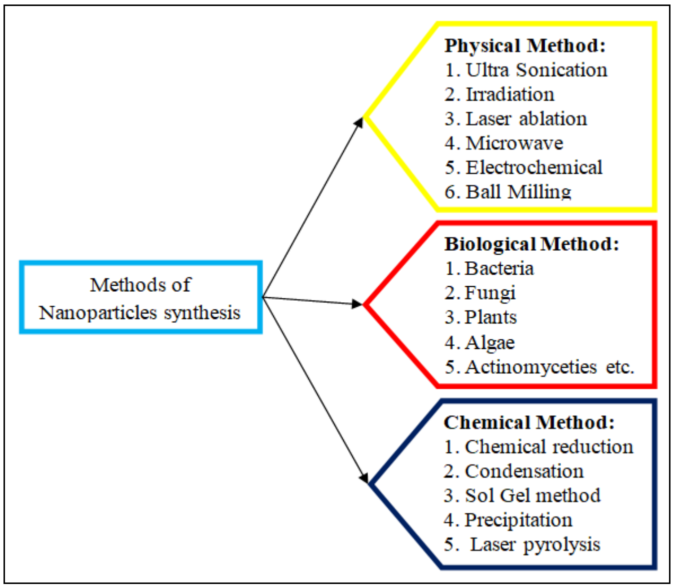

FIG. 2: DIFFERENT TYPES OF METHODS USED FOR NANOPARTICLES SYNTHESIS

Biosynthesis of Nanoparticles: The synthesis of nanoparticles by green approach is an easy, reasonable and environmentally friendly method involved different types of natural sources like plants, fungi, algae, bacteria and yeast that have potential to produce nanoparticles at extracellular as well as intracellular level. In the biosynthesis of nanoparticles from microorganisms grow in a suitable growth medium. After a proper period of incubation mycelia of fungi wash with sterilized distilled water for 4 to 5 time to remove medium from biomass and transfer in sterilized distilled water and incubated for an appropriate period of incubation **Fig. 3**. After incubation flask contains fungal mat filter again and supernatant transfer in another sterilized flasks, add metal and incubated for a suitable duration or until the visual color is changed [25, 26]. There are different types of metal shows a variety of color change during nanoparticles synthesis like from pale yellow to pinkish indicate the formation of a gold nanoparticle, pale yellow to brownish color is the formation of silver nanoparticles and whitish yellow to yellow color result in the formation of manganese and zinc nanoparticles [27, 28]. The titanium dioxide nanoparticles indicated by a change in color from purple to white [29].

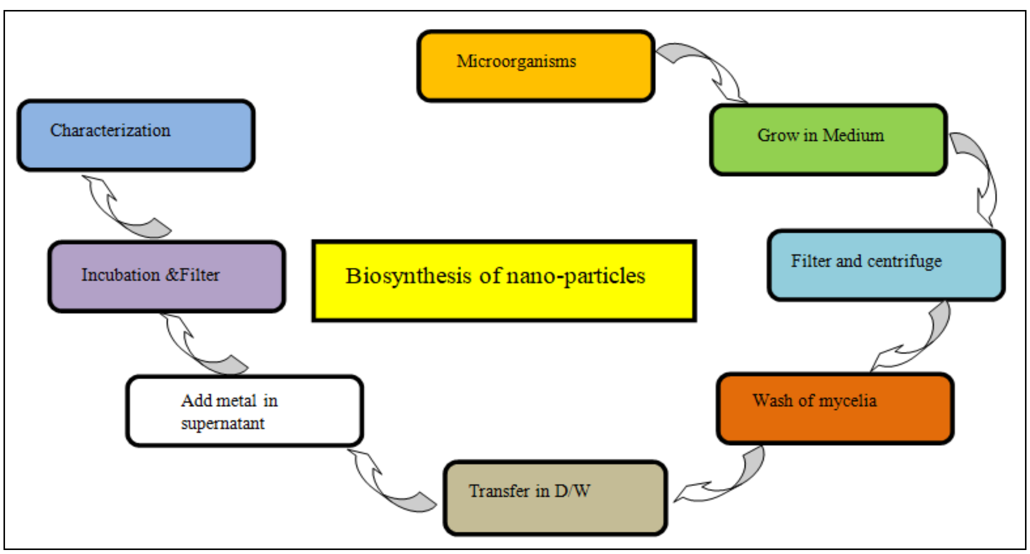

FIG. 3: BIOSYNTHESIS OF NANOPARTICLES

Nanoparticles Synthesis by Plants: The biosynthesis of nanoparticles by plants is free from toxic compounds and also provides natural capping agents [30]. Different parts of plant *viz.*, leaves, bark, stem, shoots, seeds, roots, twigs, peel, fruit, seedlings, tissue cultures have confirmed the potential for synthesis of nanoparticle [31]. The methanol extract of *Emblica officinalis* has the possibility to synthesized zinc oxide nanoparticles and also have significant antimicrobial activity against *Bacillus subtilis, Streptococcus pneumonia, Staphylococcus epidermidis, Klebsiella pneumonia,* *Salmonella typhimurium*, *Escherichia coli* and fungal pathogens such as *Aspergillus niger* and *candida albicans* [32]. The information also exists for biosynthesis of gold nanoparticles from plants used in pharmaceutical, separation sciences and biomedical purposes [33, 34, 35, 36].

The plant extract of *Emblica officinalis*, *Terminalia catappa* and *Eucalyptus hybrids* were also examined for the synthesis of silver nanoparticles and observed under UV-Visible spectroscopy, XRD, transmission electron microscopy (TEM), energy diffraction and X-ray to confirm capping over silver nanoparticles. Due to the capping of bio-molecules, these can be used in drugs delivery system [37]. Raghad *et al*., 2016 [38] biosynthesized titanium dioxide nano-particles (TiO_2 NPs) from *Curcuma longa* aqueous and observed their activity on growth, sporulation and pathogenicity on *Fusarium graminearum* and compared with commercial available nanoparticles. Vasudeo *et al*., 2016 [39] also developed nano-particles by using leaf extract of *Coriandrum sativum* L. belongs to family Apiaceae which contains phenolic compounds and flavonoids which shows better antioxidant, anti-diabetic, anti-mutagenic, anti-lipidemic and anti-spasmodic activities [40, 41, 42, 43]. A wide-range of plants reported to synthesize metal nano-particles are also given in **Table 1**.

TABLE 1: LIST OF BIOSYNTHESIS OF NANO-PARTICLES BY PLANTS

Plants	Size (nm)	Plant part	Metal	Reference
Matricaria chamomilla	60-65	Whole plant	Ag	44
Nepenthes khasiana	50-80	Leaves	Au	45
Phlomis	25	Leaf	Ag	46
Cucumis sativa	10-50	Leaf	Ag	47
Diospyros ferrea	70-90	Whole plant	Au	48
Cocous nucifera	22	Inflorescence	Ag	49
Argyreia nervosa	20-50	Seed	Ag	50
Magnolia kobus	40-100	Leaves	Cu	51
Ziziphora tenuior	38	Leaves	Ag	52
Brassica rapa	16	Leaves	Ag	27
Pogostemon benghalensis	13-15	Leaves	Ag	53
Swietenia mahogani	18-24	Fruit	Au-Ag	54
Citrus sinensis	10-35	Leaves	Ag	55
Memecylon edule	20-50	Leaves	Ag	56
Aloe vera	70-192	Leaves	Ag	57
Rosa hybrida	10	Petal	Ag	58
Eucalyptus camaldulensis	110-250	Leaves	Ag	59

Nanoparticles Synthesis by Fungi: The production of nanoparticles by fungi is a rapid and economic approach due to their easy growth, higher bioaccumulation and simple downstream process for extraction of desired products. These microorganisms produce some enzymes and protein that can act as a reducing agent for the synthesis of nanoparticles from metals. The silver nanoparticles synthesis by fungal strain *Arthroderma fulvum* had a diameter of 15 nm showed antifungal activity against *Candida* spp., *Aspergillus* spp., and *Fusarium* spp. [60] The endophytic fungal strains GX2, GX3 and ARA biosynthesized gold nanoparticles and their size were analyzed by UV-Visible spectroscopy, TEM, FTIR and observed its cell toxicity nanoparticles against a cancer cell line [61]. The nanoparticles production was also reported from *Pleuritus spp.* when it was challenged to grow at $FeSO_4$ solutions for 72 h [62]. Shelar *et al*., 2014 [63] also observed mycosynthesis of silver nanoparticles from *Fusarium semitectum* in range of 1-50 nm. The silver nanoparticles synthesized by *Fusarium semitectum* display significant antibacterial activity against *K. pneumonia* and *P. aeruginosa*.

Fusarium oxysporum has the ability of gold nanoparticles synthesis in 3h that were characterized in a range of 18-21 nm, and their effect was observed in seed germination which showed significant role to enhance seed viability in the field of agriculture [64]. The *Neurospora crassa* fungi produced silver nanoparticles at the different optimized condition of temperature and pH that improves storage efficiency of particles without any effects on its unique size [65]. Raliya and

Tarafdar, 2014 [66] synthesized different types of metal nanoparticles like zinc (Zn), magnesium (Mg) and titanium (Ti) by using *Aspergillus flavus, Aspergillus terreus, Aspergillus tubingensis Aspergillus niger, Rhizoctonia bataticola, Aspergillus fumigatus,* and *Aspergillus oryzae.* TiO_2 nanoparticle biosynthesized by fungal strain *Aspergillus flavus* was evaluated for their effect on the mung-bean plant by spray TiO_2 nanoparticles in the shoot, root, root nodule, chlorophyll content, and total soluble leaf protein. The TiO_2 nanoparticles may be applied as plant nutrient to increase crop production [67]. An endophytic fungi *Alternaria sp.* obtained from plant *Raphanus sativus* was displayed synthesis of silver nanoparticles and had anti-bacterial activity against human pathogenic bacteria [68]. List of some other fungi that play an important role in the production of metal nanoparticles given in **Table 2**.

TABLE 2: LIST OF BIOSYNTHESIS OF NANO-PARTICLES BY FUNGI

Fungi	Size (nm)	Localization	Metals	Reference
Aspergillus niger	73.58-106.8	Extracellular	TiO_2	69
Alternaria sp.	4-30	Extracellular	Ag	70
Rhizopus nigricans	7-20	Extracellular	Ag	71
Pencillium sp.	25	Extracellular	Ag	72
Pestaloptiopsis pauciseta	123-195	Extracellular	Ag	73
Fusarium oxysporum	-	Extracellular	Au-Ag	74
Candida utilis	-	Intracellular	Au	75
Verticillium sp.	25±8	Extracellular	Ag	76
Penicillium sp.	100	Extracellular	Ag	77
Fusarium oxysporum	10-50	Extracellular	$SrCO_3$	78
Trichoderma viride	5-40	Extracellular	Ag	79
Aspergillus versicolor	20.5±1.82	Extracellular	Hg	80
Fusarium oxyporum	4-5	Extracellular	BT	81
Rhizopus arrhizus, Trichoderma gamsii	20-30	Extracellular	Ag	82
Trichoderma aspercellum	13-18	Extracellular	Ag	83
Aureobasidium pullulans	29±6	Intracellular	Au	84
Neurospora crassa	32	Extracellular	Au	85
Rhizopus stolonifer	1-5	Intracellular	Au	86
Aspergillus flavus	120	Extracellular	Ag-Au	87
Fusarium sp.	100-200	Intracellular	Zn	88
Penicillium chrysogenum	5-100	Intracellular	Au	89

Nanoparticles Synthesis by Bacteria: There are different types of bacteria that are used for the production of nanoparticles which are isolated from diverse habitat. In the past few years, possibilities of synthesis of nanoparticles by bacteria are investigated because of ease in handling, economical and nanoparticles produced by these microorganisms are free from the toxic compound. The immense range of microbes resources is present in nature that can be used for the synthesis of nanoparticles [90, 91].

The bacterial strain *E. coli* isolated from urine sample synthesis silver nanoparticles and exhibits significant antibacterial activity against *Bacillus subtilis* and *Klebsiella pneumonia* [92]. The bacterial strains isolated from oil-contaminated soil sites also have the potential to produce silver nanoparticles synthesis at 85 °C and pH value 7.0 [93]. Seshadri *et al*., 2012 [94] studied marine bacterium *Idiomarina* sp. PR58-8 isolated from a soil sample collected from banks of Mandovi River in Goa, India and provides exposure to silver nitrate for 48 h for nano-particles synthesis. Similarly, marine bacteria *Pseudomonas aeruginosa* isolated from marine cost was treated with silver nitrate solution for 24 h at room temperature and observed size and nature of synthesized nanoparticles by various techniques.

The thermophilic bacterial strain *Bacillus sp.* AZ1 isolated from hot spring in Iran and identified by 16S rRNA that showed similarity to *B. licheniformis*. The extracellular biosynthesis of nanoparticles by this bacterium was confirmed by a change in color and characterized by UV-visible spectroscopy and also observed their antibacterial activity by disc diffusion method against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus epidermis* and *Staphylococcus aureus* [95]. There are some actinobacteria like *Streptomyces fulvissimus* are also used for the green synthesis of nanoparticles [96].

The bacterial strain *Bacillus cereus* isolated from the Gangetic plain of India was screened for silver nanoparticles in the range of 10-30 nm and probiotics microorganisms *Lactobacillus fermentum* also synthesized silver nanoparticles which have wide scope in pharmaceutical and medical science [97, 98]. The biosynthesis of nanoparticles by some bacterial strains is also given in **Table 3**.

TABLE 3: LIST OF BIOSYNTHESIS OF NANOPARTICLES BY SOME BACTERIA

Bacteria	Size (nm)	Localization	Metals	Reference
Bacillus subtilis	10-20	Extracellular	Ag	99
Bacillus niabensis	10-20	Extracellular	Au	100
Pseudomonas stutzeri	200	Intracellular	Ag, Cu	101
Arthrobacter kerguelensis	13-28	Extracellular	Pd, CdS	102
Pseudomonas stutzeri	50-150	Extra cellular	Cu	103
Shewanella sp.	12.52-18.43	Extracellular	Ag	104
Marine bacteria	>100	Extracellular	Ag	105
Klebsiella pneumoniae	20-40	Extra cellular	Ag	106
Pseudomona fluorescens	50-100	Extracellular	Ag	107
Arthrobacter nitroguajacolicus	40	Extra and intracellular	Au	108
Lactobacillus strains	10-25	Intracellular	Ag and Au	109
Rhodopseudomonas capsulate	3-10	Intracellular	Au	110
Bacillus licheniformis		Intracellular	Ag	111
Escherichia coli	10-50	Extracellular	Ag	112
Escherichia coli	-	Extracellular	Ag	113
Clostridium thermoaceticum	12-15	Intracellular	Cd	114
Pseudomonas aeruginosa and *Rhodopseudomonas capsulata*	10-20	Intracellular	Au	115
Arthrobacter sp. 61B and *Arthrobacter globiformis*	8-40	Extracellular	Au	116
Pseudomonas fluorescens	50-70	Extracellular	Au	117

Nanoparticles Synthesis by Algae: To decrease hazardous side-effects of nanoparticles synthesis by chemical and physical methods, some aquatic organisms like algae are also widely used for the synthesis of metal nanoparticles. The aquatic organisms contained bio-molecules, functional group, and enzymes present in the cell wall that's act as a reducing agent and have the ability for reduction of metals into ions [118]. The alcoholic extraction of marine red algae *Acanthophora specific* can reduce silver nitrate into silver nanoparticles within a range of 33-81 nm that was identified by infrared spectroscopy (FTIR). The silver nanoparticles synthesized by red marine algae also showed significant antimicrobial activity against pathogenic bacteria *Staphylococcus aureus, Bacillus subtillis, Salmonella sp., Escherichia coli* and *Candida albicans* [119].

Sargassum plagiophyllum examined for the synthesis of silver nano-particles and characterized by using different techniques like UV-visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR) and Dynamic Light Scattering (DLS) and antibacterial activity of nanoparticles were observed against bacterial test strain *Escherichia coli. Proteus vulgaris, Proteus mirabilis, Pseudomonas aureus, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera*, and *Enterococcus aerogens* [120]. The aqueous extract of *Caulerpa serutta* green marine algae also capable for the synthesis of silver nanoparticles and showed antibacterial activity against *Shigella sp., Salmonella typhimurium* and *Escherichia coli* [121].

The red seaweed species *C. Crispus* and *S. insignis* were furthermore observed for green synthesis of gold and silver nanoparticles under optimal condition. The *in-vitro* antitumor efficiency on Ehrlich ascites carcinoma (EAC) of biosynthesized silver nanoparticles from blue, green algae *Anabaena oryzae, Nostoc muscorum* and *Calothrix marchica* were also observed, and nano-particles produced by *Calothrix marchi* showed maximum activity against EAC [122]. *Chlorella pyrenoidusa* was experiential for the synthesis of gold nano-particle [123]. Omar *et al*., 2017 [124] synthesized silver nano-particles by using *Laurencia papillosa* and scrutinized their antimicrobial efficacy against different types of bacteria and fungi. Similarly, *Padina pavonica* produced extracellular gold nanoparticles in a small duration of 24 h, and antibacterial activity of these nanoparticles was observed against *Escherichia coli* and *Bacillus subtilis* [125]. The red algae *Amphiroa fragilissima* have a significant potential for synthesis of eco-friendly silver nanoparticles and display antibacterial activity against *Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, S. aureus* and *Pseudomonas aeruginosa*. The copper nanoparticles obtained brown algae *Sargassum polycystum* were reported for anticancer as well as antibacterial activity against test bacterial strain [126].

The synthesis of gold nanoparticles from brown algae *Padina tetrastromatica* was observed for their anticancer activity against human lung cancer (Hep92) and liver cell line A549 [127]. There are some other species of algae that produced nano-particles as shown in **Table 4**.

TABLE 4: LIST OF BIOSYNTHESIS OF NANO-PARTICLES BY SOME ALGAE

Organisms	Size (in nm)	Metal	Reference
Gracilaria corticata	45-57	Au	128
Sargassum muticum	5-15	Ag	129
Calothrix algae	30-120	Au	130
Ecklonia cava	30	Au	131
Padina boeregeseni	43.3	Ag	132
Garcinia mangostana	32.96 ± 5.25	Au	133
Padina gymnospora	25-40	Ag	134
Caulerpa racemosa	5–25	Ag	135
Turbinaria conoides	14-26	Ag	136
Spirogyra sp.	40-80	Ag	137
Spirogyra submaxima	20-30	Au	138
Cystoseira baccata	8.4 ± 2.2	Au	139
S. myriocystum	36	Zn	140
Turbinaria ornata	7-11	Au	141
Osmundaria obtusiloba	10 -20	Au	142
Turbinaria conoides	6 to 10	Au	143

Techniques used in Characterization of Nano-particles: For characterization of nanoparticles wide range of techniques like X-ray diffraction, X-ray Photoelectron Spectroscopy, Atomic Force Microscopy, Fourier Transform Infrared Spectroscopy (FTIR), UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) are used [30]. UV-spectrophotometer can detect the change in color of solution indicating the reduction of metals into ions which occur due to excitation of surface Plasmon vibration.

For quantitative and qualitative analysis of organic and inorganic nano-particles Fourier Transform Infrared Spectroscopy (FTIR) can be used to recognized chemical bonds between molecules and detecting the functional group present in compounds. The morphology and size of nanoparticles will be resolve by TEM, SEM, AFM and X-ray diffraction is used for determination of crystalline of the compound. Therefore, it provides a unique fingerprint of crystal present in the sample. Dynamic Light Scattering technique is help to observe particles size distribution in sample [144-147].

Applications: Scientist and researcher show great interest in the synthesis of nano-particle from different biological resource due to their unique properties which can be used in the wide area of electronics, drug delivery, sensing, pharmaceuticals, cosmetics, food and beverages, agriculture, surface coating, polymers, *etc*. **Fig. 4.**

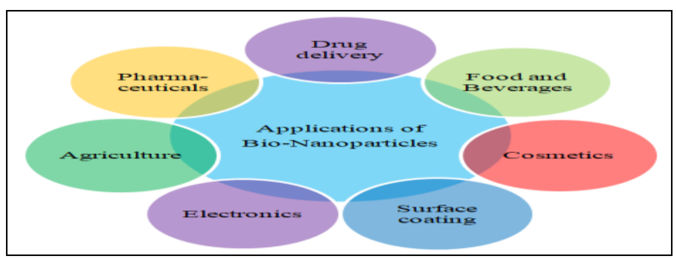

FIG. 4: APPLICATIONS OF BIO-SYNTHESIZED NANOPARTICLES

The nanoparticles synthesized by fungi have been extensively studied in recent due to their pharmaceutical potential. The extracellular silver nano-particles synthesis by the fungal strain *Candida albicans* was observed for significant antibacterial activity against the *E. coli* and *S. aureus* [148]. Similarly, silver nanoparticles synthesized by endophytic fungi *Alternaria* *tenuissima* isolated from *Punica granatum* showed broad-spectrum antimicrobial activity against the *Escherichia coli, Bacillus subtilis* and *Salmonella typhimurium, Enterococcus sp., Klebseilla pneumonia* and showed antifungal activity against *Candida albicans, Candida kruezi*, and *Candida glabrata* [68]. The gold nanoparticles synthesized by the marine bacteria *Enterococcus sp.* display unique anticancer activity against the HepG2 and A549 cancer cell line [127]. The silver nanoparticles obtained from a plant extract of *Melia dubia* showed insecticidal activity against the 4th instar larvae of *Culex qinquefasciatus* [149]. Some other studies observed that the biologically synthesized nano-particles of Pt and Pd possess vital catalytic activities and can be used as catalysts in fuel cell technology to control redox reactions and de-halogenations reactions [150, 151]. Due to their immense properties in healthcare, environment, agriculture the nanoparticles synthesis by the natural sources is an unexplored and new field that shows great potential in the Science and Technology field.

CONCLUSION: The living organisms especially microorganisms has a vast potential for production of nanoparticles which have wide application in different fields of science and technology. This review emphasized on the recent research in synthesis of metals nano-particles, their applications and methods. Now day's a number of microorganisms like bacteria, fungi, algae, actinomycetes are widely used for synthesis of nanoparticles because of their easy, cost-effective, non-toxic, eco-friendly and commercially economic importance as compared to physical and chemical methods which involve toxic material that are not only dangerous for living organisms also cause environmental effects.

The biological synthesis of nanoparticles is under in developing stage. Therefore, further research in the field of nano-particles synthesis by living cell is needed for understanding the better biological and molecular mechanisms of reaction chemical composition and shape size, *etc*. which can show great potential in the field of Biotechnology. The future research in the area of nano-particles by green synthesis play an essential role in the field of chemistry, medicine, agriculture and electronics related industries, *etc*.

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