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Biogenic Nanoparticles From *Allium Sativum* And Its Bioactives Applications

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ABSTRACT: Recent development in the synthesis of nanoparticles relies on the use of green chemistry with safe, biocompatible materials to manufacture composites, structures, and formulations based on nano for specific applications. The basic goal of green nanoparticle synthesis is to make use of the element's specific physicochemical and biological properties by linking bioactive from prokaryotes and eukaryotes for applications in various basic and applied science fields. Biogenic methods have the potential to provide advanced strategies for surface/material modification in the increasing carbonaceous materials market.

Garlic is an extensively studied herb known for possessing a wide variety of bioactive. The major proportion of garlic bioactive is organosulfur or thiol based organic compounds that have numerous biological and therapeutic activities and thus target biogenic nanocomponents and formulations for synthesis. There is, however, very scant literature on its use in the agriculture sector. The present review focuses on the synthesis of garlic related nanoparticles and their applications across various scientific fields, and their potential environmental implications when used in agriculture.

Keywords: Garlic extract, biogenic nanoparticles, nanoformulation, nano pesticide, allicin antimicrobial, MRSA, drug delivery, functional fabrics

1. INTRODUCTION:

Garlic is a well-known herb, known from ancient times as *Allium sativum* and renowned for its culinary, flavoring, seasoning, nutraceutical, medicinal and insecticidal properties. Garlic is the second-largest growing crop worldwide after onion, and China is the leading garlic and garlic based producing country, accounting for 80% of global production. All preparations against various health problems are well-practiced and recorded amongst different folk cultures and literature (Block 1985; Kamel and Saleh, 2000). The health benefits of garlic derive from the synergistic activity of present complex chemical components. It is a rich source of various mineral components such as potassium, phosphorus, sulphur, zinc, selenium, germanium, major amino acids, and moderate amounts of vitamin A and C (Josling 2005; Suleria *et al*., 2015).

Garlic is a rich source of bioactive especially organosulfur compounds and thiols which make up to 2.3% of the total nutrition composition (Kamel and Saleh, 2000; Suleria *et al*., 2015). Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate or S-allyl cysteine sulfoxide) the

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most abundant thiosulfinate (70% w/w) found in fresh garlic is an unstable volatile molecule produced by allinase based enzymatic reaction from its precursor amino acid (Agarwal 1996; Rybak *et al*., 2004). Ajoene, on the other hand, an organosulfur compound found in garlic, is a molecule that is significantly stable and biologically active and is documented for its deep antimicrobial activity which specifically inhibits quorum sensing in pathogenic bacteria such as *Pseudomonas aeruginosa* (Jakobsen *et al*., 2012; Silva *et al*., 2018). The remaining important bioactive include water-soluble S-alkyl cysteines, hydrophobic ajoenes, thiosulfinates, vinyl dithiins, oil-soluble allyl sulfides such asdiallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS), etc., (Lancaster and Shaw, 1989; Zheng *et al*., 2013; Nakamoto *et al*., 2019).

Garlic oil or its emulsified formulations are known to have a wide range of antimicrobial and anti-biofilm activity on several multidrug-resistant clinically relevant bacterial species and could therefore be considered as natural antibiotic agents (Zheng *et al*., 2013). Many authors have reported the antibacterial, antifungal, antiparasitic, antiviral, the amoebicidal activity of garlic extracts and their major bioactive allicin (Cavallito and Bailey, 1944; Polat *et al*., 2008; Fujisawa *et al*., 2009; Perelló *et al*., 2013; Zalepugin *et al*., 2015; Müller *et al*., 2016; Torpol *et al*., 2018). Garlic thiols or organosulfur bioactive are potential therapeutic agents with a wide range of biological activities and are stated to be antithrombotic, antiatherosclerotic, antihypertensive, lipid-lowering, immunomodulatory, antioxidant, antidiabetic, and anticancer (Block, 1985; Agarwal, 1996; Lawson, 1998; Rivlin *et al*., 2006; Chu *et al*., 2013; Salehi *et al*., 2019). Many studies have been undertaken on the role of chemical constituents in garlic and their therapeutic use (Lawson, 1998; Antony and Singh, 2011; Masum *et al*., 2019; Zhu and Zeng, 2020). Experimental evidence has shown that the sulfides contained in garlic oil, function as good fungicides that cause fungal hyphae causing cytoplasmic leakage and inhibiting main metabolism proteins during the cell cycle (Li *et al*., 2014).

Nanotechnology implies the use and manipulation of nanometer-size materials (1-100nm) and their applications. Garlic extracts and they are isolated bioactive has long been used as a constituent of the biogenic synthesis of nanoparticles due to their unique properties suited to various applications. Due to its high lipophilic nature, volatility, strong odor, and less physicochemical stability, the widespread use of garlic oil are restricted (Corzo-Martínez *et al*., 2007; Fahmy and Mamdouh, 2018). Therefore, technologies are required which could preserve their beneficial properties and thus increase their functional utilities. To boost their industrial applications, garlic oils may be developed into nanoscale microemulsions by reducing their normal dosage levels (Sha *et al*., 2017). Garlic based NPs were mainly exploited in the therapeutics fields and to a lesser extent in agriculture, food, and related area. There is an increasing need for sustainable, biocompatible, benign technologies to be implemented in various science fields with minimal environmental implications. The indiscriminate use of chemical fertilizers, insecticides, and pesticides in the field of agriculture has decreased soil productivity as well as degraded environmental quality. With this context, there is a growing interest in using the wide spectrum of biological activity of plant phytoconstituents such as garlic and its extracts as a source of agents to counter many phytopathogens that pose threat to agriculture production. The current review focuses on the processing of garlic extracts and they are bioactive for the synthesis of nano-based composites, structures, and formulations for use in various fields to highlight their beneficial role.

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2. GARLIC BASED BIOGENIC NANOPARTICLES AS ANTIMICROBIAL AND THERAPEUTIC AGENTS, SYNTHESIS, AND APPLICATIONS

Garlic extract or its isolated bioactive act as excellent reducing or capping agents during the synthesis of several metal and metal oxide-based nanoparticles. This is because garlic extract hosts an array of phytochemical constituents primarily organo sulfur-based compounds that have an advantage over using other plant-based bioactive in chemical interactions with metal or metal oxide components. The major advantage of using nano-based formulations is their ability to release active ingredients gradually and sustainably to the intended site. The antibiotic activity of garlic extract is mainly attributed due to the presence of the abundantly present dithiosulfinate viz allicin, which has the modifying properties of sulfhydryl and thus inhibits major sulfhydryl-containing enzymes in bacterial cells causing cell death (WILLS, 1956). When used as an antimicrobial agent the bactericidal efficacy of nanoparticles also depends mainly on the membrane structure between gram-positive and gram-negative bacteria (Kim *et al*., 2007). Allicin also reportedly inhibits RNA synthesis and lipid biosynthesis, causing damage to the cell wall in many bacteria (Feldberg *et al*., 1988; Ghannoum, 1988). Microbial cells continue to colonize on biological and non-biological surfaces, enclosed by extracellularly secreted biofilm-forming polysaccharide matrices (Lebeaux *et al*., 2014). Whether suspended single-cell colonies or clusters of cells released from pathogenic biofilms in biological systems, mainly in bloodstreams pose a significant threat to host organisms' survival (Girish *et al*., 2019). Such species often continue to develop resistance to multiple drugs such as antibiotics or chemical agents for many growth cycles, and novel methods are needed to replace traditional treatment against these recalcitrant strains of biofilm. Phytoconstituents such as garlic extracts with proven biological activities are the first choice for the synthesis of nanoparticles with enhanced functionalities in green chemistry (Majumdar et al., 2019). Several authors reported crude garlic extracts, aged garlic extracts, garlic oil, and garlic bioactive in the remediation of multiple multidrug-resistant bacterial strains and fungi. (Table-01)

MRSA (Methylene- resistant *Staphylococcus Aureus*) are known to be the main pathogens acquired in hospital and has been associated with nosocomial infections that establish a resistance to multiple antibiotics due to their mecA (methicillin resistance gene) genes that give decreased sensitivity to β-lactams (Archer, 1998; Itou *et al*., 2000; Tenover and Goering, 2009). In research studies, solgel based nanoparticles loaded with garlic extract were used for determining antibiofilm activity against multidrug-resistant *Staphylococcus aureus* (MRSA- Methicillin-Resistant *Staphylococcus aureus*). Such nanoparticles were able to release the active constituents of garlic gradually and sustainably in a slow and sustained manner by penetrating and disrupting biofilm formed by MRSA and thus exhibit strong antimicrobial activity (Girish *et al*., 2019). It may find applications where bacterial biofilms are produced in the treatment of chronic infections. The mechanism involved is based on the interference of allicin present in garlic extracts, in the synthesis of intercellular bacterial polysaccharide adhesion (PIA) involved in the formation of biofilm as studied in *Staphylococcus epidermidis* (Cruz-Villalón and Pérez-Giraldo, 2011). Recent studies have shown that allicin interferes with transcriptome rates in staphylococcus aureus cells by inducing high thiol specific oxidative and sulfur stress leading to protein damage and widespread proteome S-thioallylations (Loi *et al*., 2019). This is in line with another related study where allicin is found to decrease bacterial adhesion and exopolysaccharide secretions, plausibility by decreasing various virulence factors such elastase, exotoxin A, rhamnolipids and factors involved in quorum sensing as reported in *Pseudomonas aeruginosa* (Lihua *et al*., 2013). In another analysis on anti-biofilm activity extracts of garlic clove capped silver nanoparticles (garlic-AgNPs) of size 10-50nm and with monodispersive nature were

synthesized. The garlic-AgNPs showed deep antibacterial activity and anti-biofilm activity against MRSA and *Pseudomonas aeruginosa* which are of clinical importance. These eco-friendly nanoparticles have exhibited strong antihelminthic (earthworm) and anti-cancerous (against breast cell lines) properties (Vijayakumar *et al*., 2019). Nanomaterials such as carbon dots have been recently attempted for their application in drug delivery systems due to their easier synthesis, low toxicity, and high biocompatibility. Within this overview segment on bioimaging, the properties of carbon dots are clarified within the brief. Carbon dot-coated alginate beads (CA-CD) were synthesized as a drug delivery mechanism in the study designed to build a smart drug delivery system against MRSA (Majumdar *et al*., 2016). These were then filled as model drug system (CA-CD-GE) with garlic extract that included allicin.

While applied against MRSA, the CA-CD-GE system was able to result in a controlled release of active components in a pH-dependent manner increasing its therapeutic efficiency. It was also demonstrated that the active component release was also dependent on MRSA concentrations, wherein the concentration of MRSA increases the pH of the surrounding media decreases. Therefore, these stimulating smart drug delivery systems based on CA-CD-Ge may find their potential in systems where the pH of the pathogen is a determinant factor for infection as found in MRSA.

A comparative study on immunomodulatory and antiparasitic (*Eimeria steidai*) effects against hepatic coccidiosis in rabbits using garlic-based zinc nanoparticles (ZnNPs) and pure garlic extracts were made (Mohi-Eldin *et al*., 2018). Garlic based ZnNPs showed a significant but equal amount of antiparasitic effect compared to their crude counterparts. In another report, poly dispersive silver nanoparticles of size 7.3±4.4 nm were synthesized using aqueous garlic extracts as a part of green synthesis in the presence of sunlight as a natural catalyst by (Rastogi and Arunachalam, 2011). Garlic extracts had both acted as green reduction agents and capping agents. It has been shown that the synthesized AgNPs have good antibacterial potential against several Gram-positive (Staphylococcus aureus) and Gram-negative bacteria (Pseudomonas aeruginosa). In a similar attempt to use garlic extracts (Bouqellah et al., 2019), AgNPs synthesis showed strong antibacterial activity against vaginal pathogens such as Pseudomonas aeruginosa and Streptococcus pneumonia was observed. Chitosan a water-soluble, biodegradable and linear copolymer is composed of N-acetyl-D-glucosamine and D-glucosamines in different proportions, is extensively used for the synthesis of biocompatible nanoparticles and are prospective carriers for delivery of a broad category of drugs. To study the efficacy of nanoparticles against methicillin-resistant *Staphylococcus aureus* (MRSA) causing skin infections and other antimicrobial properties in mice model, a nanoformulation of allicin with AgNPs were attempted (Hoseini Alfatemi *et al*., 2014). The synergistic effect of AgNPs with allicin in combination as a nanoformulation against skin infections in mice model caused by MRSA demonstrated their antimicrobial efficacies, thus could be promising agents to combat multidrug-resistant strains and better alternatives for antibiotics. Of the various nanostructures and carriers for targeted drug delivery, polymer nanocomposites are in wide application. Polymer nanocomposites (PNCs) are composed of a polymeric nano matrix usually filled with materials of nanoscale dimensions. PNCs are commonly used in targeted drug delivery systems because of their specific capability to catch drug candidates as encapsulated particles or surface bearing forms of active molecules. These properties in effect improve the stability of the target molecule as well as its half-lives and are ideal candidates for dynamic drug delivery (Mishra et al., 2010). In a study, garlic oil-based nanoparticles were synthesized for their potential use as nano

biotics. Polymeric composite nanoparticles containing garlic oil and poly (lactic-co-glycolic) acid (PLGA) were prepared by a single emulsion/ solvent evaporation method. Their antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus* showed enhancement in inhibition by 70-78% compared to bulk garlic oil (Fahmy and Mamdouh, 2018). Phytoconstituents when used in combination are known to exhibit profound antibiotic and antifungal activities. In a study, zinc oxide nanoparticles (ZnONPs) were synthesized using aqueous extracts of garlic, rosemary, and basil plants for potential use as antibacterial agents. The plant-based ZnONPs exhibited enhanced antioxidant and efficient antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* bacterial strains compared to their chemically synthesized counterparts (Stan *et al*., 2016). A combination of chemical and green synthesis techniques was used for the manufacture of cobalt ferrite and silver doped cobalt ferrite nanoparticles using sol-gel auto combustion technique with extracts of *Ocimum sanctum* and *Allium sativum* (Mahajan *et al*., 2019). Ferrite and cobalt ferrite based nanoparticles have been highly exploited in biomedical applications due to their unique electrical, structural and magnetic properties, chemical stability and large magneto-crystalline anisotropy (Jia *et al*., 2012; Amiri and Shokrollahi, 2013; Sanpo *et al*., 2013; Amini *et al*., 2018). The produced NPs were found to possess profound antibacterial activity towards several Gram-positive bacteria compared to Gram-negative strains due to their differential interaction with nanoparticles and the cell wall structural variations among both strains. The role of sunlight irradiation as a catalyst in the synthesis of green nanoparticles is a cost-effective process and has been reported by several authors. Especially in the synthesis of AgNPs sunlight plays a vital role as evidenced by the experiments where sodium dodecyl sulphate and sunlight were involved, also it is reported to be involved in the toxicity reduction of gum arabic based polymeric AgNPs (Cheng *et al*., 2011; Bhaduri *et al*., 2013). The green synthesis was made for generating highly stable AgNPs using aqueous garlic extracts that serve as both a capping and reducing agent by exposing the mixture for 15 minutes under bright sunlight as a catalyst source. Thus, synthesized poly dispersive NPs have been stable for a long period showing effective bactericidal activity against selected Gram-positive and negative bacteria (Rastogi and Arunachalam, 2011). Gamma radiations were used as a green synthesis approach for producing gold NPs (AuNPs) which is a clean, simple, and inexpensive process where radiolysis is involved in the reduction of metal ions. The aqueous solution of fermented soybean extract along with garlic extracts as co-substrate was used as the reaction components. *Aspergillus oryze* was used in solid-state fermentation reaction to enhance the mobilization of antioxidants from soybean matrix and garlic as co-substrates by modulating the polyphenolic substances. The bioactive (mobilized polyphenols and proteins) present in the extract provided stability to the synthesized gold nanoparticles (AuNPs), with stability, and the AuNPs produced showed strong antibacterial effectiveness against several Gram-positive and negative bacteria (El-Batal *et al*., 2013).

Garlic extracts are known to impart changes in membrane conductivity as well as altering membrane permeability in fungal mycelia thereby leading to protein leakage resulting in disruption of cell membrane integrity (Chen *et al*., 2018). A study on the antimycotic activity of garlic-based silver nanoparticles was attempted by (Robles-Martínez *et al*., 2019). Dermatophytic onychomycosis fungal infection of skin was challenged by synthesized *Allium sativum* extract AgNPs (AsExt-AgNPs), which showed complete inhibition of *Tricophyton rubrum* a dermatophyte, making them more effective antimycotic candidates at all-natural concentrations of AgNps. The proposed mechanism for their antimycotic properties has been the cytoplasmic membrane disruption by garlic extract decorated silver nanoparticles against *Tricophyton rubrum.*

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Polybutylcyanoacrylate (PBCA) nanoparticles were wrapped with allicin through emulsion polymerization method in a study to test the in vitro antifungal efficacy of the garlic-active theory allicin (Luo et al., 2009). PBCA-Allicin NPs have shown successful inhibition of *Candida albicans, Cryptococcus neoformans, Trichophyton rubum, Microsporum gypseum, M. Canis* and *epidermophytone floccosum* relative to pure allicin, which may be attributed to the continued release of allicin after wrapping into PBCA NPs and their nanoscale dimensions. Liposome-based nanovesicles are interesting candidates to deliver bioactive components into food as they are known for entrapment, delivery and release of lipid, water-soluble and amphiphilic materials (Mozafari *et al*., 2008). Liposomes are also biodegradable, biocompatible and have properties of sustained release of carrier bioactive components (Neethirajan and Jayas, 2011; Neves *et al*., 2013). Liposome-based nanovesicles were developed in a study to counter Listeria bacteria in milk. Liposomes encapsulated garlic active components, as well as garlic extracts, be effective in growing the viable cell count of Listeria in milk up to 4 log cycles, thus proving technology for various food processing applications and primarily for the supply of antimicrobials (Pinilla et al., 2017).

Monodisperse AgNPs were synthesized using an extract of garlic acting as a reducing agent for studying cell toxicity and their stability in biological media in a one-pot reaction. The components of biological media are known to interfere with nanoparticles causing irreversible agglomeration and thereby causing undesired effects. The cytotoxic studies on vascular smooth muscle cells (VSMCs) and NIH 3T3 fibroblasts showed no side effects of these garlic-based AgNPs thereby paving way for their possible applications in several biomedical applications. Such NPs also demonstrated high oxidation resistance when reacted with H2O2 and good compatibility with the biologic media. Mainly allyl sulfides in garlic extracts have been attributed to organosulfur compounds for oxidation resistance of AgNPs (Von White et al., 2012). Aqueous extracts of garlic cloves as reducing/stabilizing agents were used in the synthesis of high in vitro stability and long shelf life gold nanoparticles (AuNPs) without any aggregation. FTIR (Fourier-transform infrared spectroscopy) analysis hinted at the role of S-allyl cysteine as the major component for the possible involvement in the reduction of Au3+ to Au0 and protein/amino acids as reducing agents. The synthesized AuNPs were non-toxic in cytotoxicity assays on *S.cereviciae*, thus proving to be potential candidates for applications in drug delivery (Rastogi and Arunachalam, 2012). To utilize the garlic processed wastes, a novel method of using *Allium sativum* skin extracts (garlic peel) was used to synthesize ZnO NPs (Shreya Modi and Fulekar, 2020). In this experiment, the waste garlic peel extracts were involved in the reduction of zinc chloride to zinc oxide. However, the authors did not report any biological activities of ZnO NPs. For its hepatoprotective properties, age-old garlic extracts rich in S-allyl cysteine and S-allyl mercaptocysteine are documented (Nakagawat et al. 1989). In a highly stable study, polydispersive, spherical gold nanoparticles (AuNPs) were synthesized to investigate their hepatoprotective and potential anti-inflammatory activities in conjugation with aqueous garlic extracts. The NPs showed excellent hepatoprotective activities in a CCL4 induced acute hepatic injury in a mice model as well as good Urease inhibitory action proving their role in the treatment of injury and inflammation (Ateeq *et al*., 2015). A class of nanoparticles system called solid lipid nanoparticles (SLNP) have been developed during the previous decade and are found to be better carriers of lipophilic bioactive and drugs in colloidal forms (Vringer, 1999; MuÈller *et al*., 2000). It is proven to be more efficient than traditional colloidal drug carrier systems like emulsions, liposomes and several polymeric micro or nanoparticle systems (Gulati *et al*., 1998). SLNP system could be used to efficiently synthesize garlic oil-based nanocarriers to better address the solubility issues found in traditional systems. In a study, high-pressure homogenization and ultrasound techniques were

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employed to synthesize SLNPs where garlic oil was incorporated (Wencui *et al*., 2015). This garlic oil -SLNPs showed high stability and could be stored for longer periods in lyophilized form. This, when subjected to pharmacokinetics studies before their application as an in vivo therapeutic agent, showed rapid degradation by mononuclear phagocytes before being fully eliminated by liver lipases which proved safe for the intended use.

3. GARLIC BASED BIOGENIC NANOPARTICLES IN AGRICULTURE AND AQUACULTURE

For modern-day farming, the use of synthetic pesticides is the primary option because it provides immediate results for the purpose used. Such synthetic pesticides include insecticides, fungicides, herbicides, acaricides, rodenticides, molluscicides and regulators for plant growth in synthetics as well. But indiscriminate usage of synthetic pesticides and fertilizers in cultivation has resulted in soil quality degradation and fertility even resulting in harmful environmental implications. When compared to other sectors like biomedical, materials, and energy sectors the usage of nanotechnology in agriculture has received marginal attention (Marchiol, 2018). The use of sustainable technologies in modern agriculture is the need of the hour and nanotechnology provides ample opportunities due to its targeted delivery of active compounds as compared to the conventional usage where only 0.1% of chemical pesticide reaches the target (Liang *et al*., 2017). The role of nanotechnology applications in agriculture is reviewed by several authors (Chhipa, 2017a, b; Marchiol, 2018; Singh *et al*., 2019). Plant-based benign biocompatible biodegradable biogenic nanoparticles as a part of green synthesis have resulted in the development of innumerable nano-based products that have shown promise for their safe usage to cater to the agriculture and food processing sector. Crude garlic extracts or their formulations are believed to prevent pest activity from primordial time.

Experimental evidence reported by several authors has proved the efficacy of garlic extracts and garlic essential oils against different agricultural pests (Park and Shin, 2005; Dąbrowski and Seredyńska, 2007). Steam distilled garlic clove extracts were tested against two-spotted spider mite (Tetranychus urticae) in a laboratory experiment to verify its effectiveness as an acaricidal agent. After steam distillation, the modified garlic such as vinyl dithiin, diallyl disulphide, diallyl trisulphide could plausibly contribute to their effective biological activity against T.urticae (Attia *et al*., 2012). Thus, garlic extracts or bioactive components while utilized for the synthesis of nanoparticles and formulations are expected to show a magnified impact on the proposed applications. In an experiment to investigate the insecticidal activity of PEG (Polyethylene Glycol) encapsulated garlic essential oil, a nanoformulation was made. These were challenged against red flour beetles (*Tribolium castaneum*) infecting harvested rice. The study showed 80% mortality after 5 months of storage compared to 11% with garlic oil alone. Nanoencapsulation allows the slow and persistent release of garlic active components thereby prove to be effective candidates for eco-friendly bioinsecticides (Yang 286 *et al*., 2009). Antifungal activity of garlic extract-based green synthesized ZnO NPs, ZnO nanohybrids and chemically synthesized ZnO NPs were compared by (Arciniegas-Grijalba *et al*., 2019). The green synthesized garlic impregnated ZnO NPs, as well as garlic-based ZnO nanohybrids, showed 93% and 97% inhibition respectively of fungi namely *Mycina citricolor* and *Colletotrihum* sp. infecting coffee plants, compared to their chemically synthesized counterparts. In another study, the efficacy of garlic oil-based nanoacaricides, a nanoemulsion using the ultrasonic emulsification technique were synthesized to check their acaricidal activity. The 93.4 nm-sized garlic oil nano-emulsion was found to have high acaricidal activity against phytophageal eriophyid olive bud mite Aceria oleae Nalepa and rust-causing mite Tegolophus Hassani (Keifer) and was also found to be non-toxic in rat

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models. Garlic bioactive such as diallyl sulfide (8.6%), diallyl disulfide (28.36%), dimethyl tetrasulfide (15.26%), trisulfide, di-2-propenyl (10.41%), and tetrasulfide, di-2-propenyl (9.67%) found to contribute to acaricidal activity. (Mossa *et al*., 2018). Nanotechnology has also found its application in freshwater prawn cultivation as a part of increasing nutrition value for live food organisms for fry production. In one such study, monodispersive and stable selenium nanoparticles from sodium selenite were synthesized using garlic extracts. These NPs were used to feed *Artemia nauplii*, a Braun shrimp which in turn serves as preferred live food for freshwater prawn *Macrobrachium rosenbergii* post-larvae. Selenium also serves as an essential trace element is an integral part of the cellular defense mechanism against oxidative stress in cellular systems (Satgurunathan *et al*., 2017). The same group also reported the synthesis of chromium nanoparticles using aqueous garlic extracts to improve the essential mineral uptake and improve the nutritional quality of prawn *Macrobrachium rosenbergii*. This was then fed to Artemia nauplii brine shrimps, as this is the preferred feed for M. Rosenbergii (Satgurunathan et al., 2019). There was no toxic effect of Artemic nauplii enriched by these chromium nanoparticles and could thus be a healthy mineral nutrient substitute for sustainable prawn cultivation.

4. GARLIC BASED NANOCOMPOSITES IN FUNCTIONAL FABRICS

For biomedical applications, practical fabrics with wound healing and antimicrobial properties require an hour, as they provide essential barriers against pathogens from patient blood or body fluids. Nanoparticles and other nano-based composites are known to have significant inhibitory actions against a large variety of microorganisms. Materials such as Ag, Cu, CuO, Ti, ZnO used in nano synthesis have been used to functionalize fibers and fabrics, enhancing the consistency of conventional fabrics with new properties, thus establishing a field of intense work in antimicrobial textile production (Borkow et al., 2010; Rivero et al., 2015). Cerium oxide (CeO2) nanoparticles are found to possess properties of radical scavenging activities due to their dual oxidation state (Ce3+/Ce4+ redox switch) and hence possess unique biological activities thereby used as a potent therapeutic agent for various ailments and also in different industrial applications (Wason and Zhao, 2013). In a study, CeO2/allicin based nanocomposites were impregnated on woven and non-woven durable fabrics to study their efficacy on wound healing. A sulphur based bioactive from *Allium sativum*, the diallylthiosulfinateallicin imparted antimicrobial properties when bound to the antioxidant CeO2 nanoparticle (Thanka Rajan *et al*., 2017). Copper nanoparticles phytofabrication with an extract of garlic leaves were attempted to check their antibacterial properties. It has been found that the synthesized 100 nm CuNP is more effective against Gram-positive bacteria (Bacillus subtilis) (Joseph *et al*., 2016). Allicin-conjugated nanocellulose was prepared for use in finished fabrics in a similar attempt and tested for their antimicrobial properties (Jafary *et al*., 2015). The fabrics had good resistance towards *Stapylococcus aureus*.

5. IMAGING AND CELLULAR SIGNALING APPLICATIONS

The most studied topics in the field of bioimaging, sensing, and optoelectronic devices are natural fluorescent substances and materials (Sun *et al.*, 2016). Thanks to their high photostability, improved photoluminescence quantum yields, and molar extinction coefficients with size adjustable emission, nanoparticles such as semiconductor quantum dots are the favoured choices in such applications. However, there are serious environmental and toxicity issues involved in the usage of these quantum dots owing to their usage in organic solvents and hence restricting their direct applicability. Carbon nanoparticles or nanodots could be the preferred alternative for quantum dots due to their high-water solubility, comparable luminescence and photostability as well as high biocompatibility. In a study,

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garlic was used as a carbon source to synthesize carbon dots as a part of green synthesis by (Srivastava *et al*., 2014; Sun *et al*., 2016). To increase the photoluminescence quantum yield ethylenediamine and sodium thiosulphate was added respectively for generating Nitrogen and Sulphur rich CDs during the synthesis process. The synthesized CDs were highly stable exhibiting no signs of photobleaching on UV exposure while the CDs were found to have efficient fluorescence quenching properties in the presence of Fe3+ ions thereby could be used to detect Fe3+ ion concentration is solutions. Carbonaceous materials include carbon nanotubes which when modified or decorated with nanoparticles find wide applications in electronics biomedical and biosensor utilities wherein increasing the performance by one order of magnitude. Sidewall modification of multiwalled carbon nanotubes was achieved by eco-friendly green synthesis method using extracts of garlic. The inner walls of these nanotubes were coated with gold nanoparticles via organosulfur (allin/allicin) linkages suggesting coalescence among nanoparticles. (Srivastava *et al*., 2013). Garlic is a source of a variety of phytochemicals from thiol and Sulphur. Through a one-step reaction, graphene oxide was converted into graphene nanosheets modified by organosulphur using Allin-a garlic phytochemicals. Pre-synthesized Au-NPs were attached through allin mediated organosulfur (thiol) connections to these OS-GNS. The final nanocomposites of au-graphene find their use in electronics, sensors, and other biomedical applications (Srivastava *et al*., 2014). Gold nanoparticles are of interest in the field of biomedical and molecular imaging applications due to their quantum scale dimension imparting particular electronic and optical properties especially plasmon bands. When plasmon bands are excited with laser light they enhance the Raman cross-section of the nearby molecules to a higher magnitude thereby making the signals highly sensitive. Hence they are used in Raman Spectroscopy as Surface Enhanced Raman Spectroscopy (SERS) active substrates that find applications in both solutions or as internalized inside living cells like human fetal lung fibroblast (HFL-1) as reported by (Coman *et al*.,2014). The authors used aqueous garlic extracts to synthesize stable nanoparticles of gold that were acting as reducing and capping agents. Biochemical and molecular examination of G-AuNPs showed the existence on the surface of garlic protein coating making them non-coalescent and inert, which is a significant feature of biomedical use. Such non-toxic nanoparticles may still intracellularly maintain SERS- signaling. Carbon dots or carbon nanomaterials are biocompatible due to their tenable physicochemical properties, simple modifications, and stability, and are used in many biological applications. In an attempt of using green chemistry in the synthesis of fluorescent carbon dots, (Zhao *et al*., 2015) used garlic extracts as a precursor in the reaction. These garlic synthesized CDs showed comparable performances in terms of fluorescent properties with other fluorescent dyes and finds application in free radical scavenging and cellular imaging domain. In another study, microwaves were used to synthesize biogenic carbon nanodots using garlic extracts having superior photostability. Such biocompatible theranostic carbon nanodots which are highly water-soluble find use in bioimaging. The authors also demonstrated their powerful antioxidant properties in the macrophages (Yang *et al*., 2015).

6. GARLIC BASED NANOFORMULATIONS FOR FOOD PROCESSING, PACKAGING AND PROTECTION

The term food processing may be characterized as food preservation by different means or technologies, thereby turning food into a consumable state. (Pradhan *et al*., 2015). The term food processing includes the conversion of raw food ingredients into products that have a longer shelf life and marketable. The processing technology includes the removal of toxins, preventing microbes like fungi and bacteria, developing preservation methods thereby providing consistency in the quality of food for better marketing (Chellaram *et al*., 2014). Food packaging is a process of containing processed or raw food ingredients from external

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contaminants also maintaining their sensory quality and other attributes for the ultimate convenience of the consumer (Robertson, 2013). Current food processing and packaging industries harness the advantages of different aspects of nanotechnology and thereby pursue more environmentally sustainable strategies and contribute to food health. Food packaging manufacturers are exploring greener and more sustainable sources of natural polymers at an unprecedented pace globally due to the growing surge of waste plastics. Extensive work on turning polymer waste into usable packaging materials has led to innovative goods. In one such attempt waste plastics were utilized to synthesize multi-walled carbon nanotubes by chemical vapor deposition method for developing biodegradable packing materials (Pattanshetti *et al*., 2020). These nanotubes were used as nanofillers and blended with gelatin to convert them to nanocomposite films with good mechanical strength, decreased water and oil-soluble capacity, and antimicrobial properties. Parallelly garlic-based microparticles were synthesized using a ball milling technique and coated on gelatin multi-walled carbon nanotube (Gelatin/MWCNT-3%) composite film. Garlic microparticle coating was used to avoid the direct interaction of GMWCNT with the food as well as to impart antibacterial activity while using it as packing material. These GMWCNT nanocomposite films blended with garlic microparticles thus could be used as potential food packaging materials as the technology helps to remove waste plastics from the environment. In another study, aqueous garlic extracts and carboxymethyl chitosan were used to prepare edible crude nano-coating solutions by the ultrasonication method (Diao *et al*., 2020). The Nano-coating solution for garlic extract-CMCS was then added by soaking ready to eat spiced chicken meat to prolong the shelf life. The authors showed that this approach can effectively prevent microbial development, even delay protein breaking rate and lipid oxidation in chicken meat without altering the sensory attributes significantly. The high diallyl sulfide containing aqueous garlic extracts-CMCS nano-coating solution thus could be used as a potential meat storing agent. Active food packaging is an emerging area of research to develop new packing materials with desirable functional properties including antibacterial, antifungal and antioxidant activities. In one such study active plasticized banana flour nanocomposites were synthesized blended with different content of garlic oil to study their effectiveness in preserving roasted peanuts (Orsuwan and Sothornvit, 2018). Incorporation of garlic oil into APBNF resulted in the highest antioxidant activity as well as completely inhibited the growth of Aspergillus flavus. Garlic oil blended nanocomposites thus could be good packing agents for the storage of oily food products as reported by the authors. Synthesis of hydrocolloid based biodegradable materials have been exploited in biomedical applications where the focus is to develop biofilms with better mechanical and water-resistant properties using a combination of different polymers (García Schejtman *et al*., 2015). In a study, Gelatin-based biodegradable hydrocolloid films were obtained after blending with Ag NPs synthesized with aqueous garlic extracts that have bactericidal properties due to the presence of NPs (Fernanda *et al*., 2018). Functional foods are very beneficial and have tremendous potential in improving human nutrition, providing optimal health benefits, longevity, and improved quality of life for patients with degenerative diseases and hence formulations involved in functional foods are trending in global food industries (Kahlon and Keagy, 2003; Biesalski *et al*., 2009). Nutraceuticals or bioactive incorporated in such processed functional foods could benefit human health thereby complementing a balanced diet. In a study to improve the physical and sensory parameters of functional bread fortified with garlic oil, hybridized calcium alginate microcapsules containing nanoemulsions of garlic oil were prepared using air atomization technique (Narsaiah *et al*., 2019). Microcapsules are efficient in the controlled release of encapsulated bioactive when incorporated into formulations having non-compatible functional food components and work as biological preservatives (Narsaiah *et al*., 2014). The purpose of garlic oil incorporation in bread was to harness its health-promoting

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and antioxidant efficacy without any impact on the physical or sensory attributes of the functional bread. Some essential oils' antibacterial properties have contributed to their use as food preservatives for prolonging shelf life. A nanoemulsion formulation based on garlic, cinnamon and ginger essential oils were prepared in a study to create an essential oil-based food preservative at a ratio of 80:10:10 with a high-pressure homogenizer (Hernani and Dewandari, 2018). These were converted into nanoemulsion powder by spray drying where maltodextrin was used as a filler. The nanoemulsion powder inhibited the growth of Escherichia coli and Salmonella typhi at a concentration range of 10%-15% with minimal LC50 values. The results suggest that the nanoemulsion mixture of garlic, ginger and cinnamon could be used as potential food preservatives. Thus garlic-based nanocomposites, nanoformulations have now become important constituents of the food processing industry. Phytoconstituents such as garlic extracts, essential oils in garlic, or bioactive in garlic are one of several agents explored in the applications used to impart improvised properties.

7. GARLIC AND ITS BIOACTIVES IN MISCELLANEOUS NANO APPLICATIONS

Organic-inorganic hybrid nanostructures called "nanoflowers / hNFs" have been developed with increased catalytic activity and stability in recent years when protein/enzyme is used as organic components (Ge *et al*., 2012; Zhu *et al*., 2013). Nanoflowers have found potential applications in enzyme purification, personal health care devices, sensors for volatile gases and metal detection, environmental waste treatment, drug delivery and various other industrial processes (Shende *et al*., 2018; Huang *et al*.,2019; Wang *et al*., 2019). Allicin and copper-based organic-inorganic hybrid copper nanoflowers with high stability and catalytic activity were synthesized for studying their peroxidase-like and antimicrobial activities (Koca *et al*., 2020). These hybrid nanoflowers were also shown to exhibit antibacterial activity against fish pathogens namely *Aeromonas hydrophila*, *Vibrio parahaemolyticus* and *Lactococcus garvieae.* In biochemical studies, these nanoflowers could be an effective alternative for Fenton agent for peroxidase reaction. Antimicrobial textile coated fabrics have tremendous market potential with the tendency of peoples towards these fabrics for a healthier lifestyle and are areas of intensive study. Nanocellulose has a high volume-to-volume ratio, crystallizing potential and antimicrobial activity to some degree, strong stability against proteolytic enzymes, temperature regimes, acids and is biodegradable (Pandey *et al*., 2013).

Nanocellulose is also known for their good mechanical properties, low weight and filler load requirements, reinforcing ability based on which they found applications as reinforcing fillers in nanocomposites, adsorbents, strengthening component in paper, carriers of bioactive, drugs or genes in medicine, degradable films in the packaging and textile (Habibi *et al*., 2010). Industrial wastewater treatment is a challenge due to their complex chemical nature of the pollutants. With a high degree of aromatic groups, azo based dyes from the textile industry are the major contributors of pollutants with a 50% share among textile dyes as reported and pose a serious challenge to the environment since they are not easily degraded by conventional technologies (Lee *et al*., 2006; Song *et al*., 2008). With the advent of nanotechnology, the advanced oxidation method dependent on photocatalytic properties of some nanocomposites is attempted to tackle the remediation of textile colors effectively (Song *et al*., 2008; Karimi *et al*., 2014). In one of these papers, nanoparticles based on garlic extract (Fe3O4) were synthesized using a green hydrothermal method (Khaghani and Ghanbari, 2017). These Fe_3O_4 NPs were used to synthesize silver nanocomposites again in the presence of garlic extract acting as surfactant via the green hydrothermal method with the desired Fe_3O_4-Ag nanocomposite ratios (90%:10%, 50%:50%). The synthesized Fe_3O_4-Ag

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nanocomposite exhibited applicable paramagnetic properties as well as exhibited remarkable photocatalytic degradation of three reference azo dyes under ultraviolet irradiation. Such nanocomposites may be widely used in the field of textile wastewater remediation as possible photocatalytic reagents against azo dyes.

8. CONCLUSION AND FUTURE PROSPECTIVES

A study aims to capture the applications of garlic extracts and their nano synthesis bioactive demonstrating the advantages of phytoactive to the good of mankind. The ability of bio-based nanoparticles solely depends on the effectiveness of formulations developed on an industrial scale particularly. Nanotechnology is in its early stages, given its significant application in the medical and pharmaceutical industries, and is comparatively less desirable in agriculture. Nanotechnology's full potential has yet to be recognized in the agricultural and food processing industries. It discusses numerous reports about biogenic nanoparticles using phytochemicals and their applications. These findings have failed to translate into new nano-based products, however, and the technology is always seen in caution that narrows its applicability. Studies on different nano-based products are inconclusive from an environmental viewpoint, and questions about the fate of applied nanomaterials. The potential phytotoxicity of nanomaterials is previously reported by many authors where bioaccumulation and higher bioaccessibility are the negative implications (Jampílek and KráL'Ová, 2015; Pacheco and Buzea, 2018). Many nanomaterials are engineered to release active molecules on an ongoing basis, and thus remain in the environment for longer periods. This in effect affects the nanomaterial's physicochemical properties, making them leaky to biological systems or climate, thereby creating health concerns (Abid *et al*., 2020). Thus, it is emphasized to conduct rigorous field trials before implementing the usage of such nanoparticles before adopting in the agrarian sector. Commercialized nano-based goods are often rarely owned during large-scale manufacturing due to their possible environmental consequences, economic viability considerations, and the costs involved. Garlic extracts and their bioactive volatiles are widely used in both traditional forms and as nano-based products for their possible benefits across the scientific fields. Thus, more focus needs to be put on using biocompatible, biodegradable nanocarriers, composites, or formulations in agriculture. Since most of the garlic bioactive are volatile and with short half-lives, they are promising candidates while used as nano pesticides, acaricides, bactericides, insecticides, or fungicides. More emphasis needs to be placed on developing strategies for large-scale production with the least environmental impact. Garlic extracts are potential additives in food processing industries, with their inherent antimicrobial properties. Where the construction of a nano based biodegradable packaging, material mixed with garlic bioactive needs the utmost care to avoid possible leakage of active substances on the stored food. Although bioactive based on garlic such as allicin and ajoene in their nano form are studied primarily using specific cell lines in therapeutic use, they have shown themselves to be potential drug candidates against various ailments. Such experiments have shown less toxicity effect in cell cultures, but they need to decode their possible pharmacokinetics, toxicokinetic, and toxicodynamic findings when using applications in situ. There are wide opportunities for applications based on biogenic nanotechnology, and for industries. Garlic-based constituents may be the best option to grow nano molecules, with their variety of chemical moieties that communicate with biological and chemical components benefiting both nature and humanity.

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Table 01: Garlic Based Extracts And Their Bioactive Agaisnt Mrsa And Miscellaneous Microbes

Sl. No	BIO ACTIVE	BACTERIA	FUNGI	EXPERIMENTAL MODEL/ SOURCE USED	PREDICTED MECHANISM	REFERENCE
1	Fresh Garlic Extracts	MRSA, *Pseudomonas aeruginosa*	*Candida albicans*	Clinical isolates and type cultures	Synergistic antimicrobial effect of aged garlic extract with fungistats and antibiotics on bacterial and fungal growth	(Li *et al*., 2015)
2	Garlic Extract	MRSA Skin		Wistar Albino Rats		(El-Gayar *et al*., 2016)
3	Fresh Garlic Extracts	MRSA & non-MRSA		Clinical isolates and type cultures with the chemopreventive and chemotherapeutic approach	Suggested interference and downregulation of garlic bioactives against biofilm and quorum sensing associated genes.	(Rattha wongjirakul and Thongkerd, 2016)
4	ALLICIN	MRSA		Clinical isolate, type cultures and mutants of *S.aureus*	Allicin induced S-thioallylations, thiol specific oxidative and sulfur stress, interference with cell wall homeostasis and related genes	(Loi *et al*., 2019)

Abbreviations: DAS-diallyl sulfide, DADS-diallyl disulfide, DAT-diallyl trisulfide, DATS-diallyl tetrasulfide, MRSA-Methicillin resistant *Staphylococcus aureus*

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