

REVIEW

Open Access



# Current trends in antimicrobial activities of carbon nanostructures: potentiality and status of nanobiochar in comparison to carbon dots

Kulathi Nishshankage<sup>1,2</sup>, Andrea Breverly Fernandez<sup>1,2</sup>, Shiran Pallewatta<sup>1</sup>, P. K. C. Buddhinie<sup>2</sup> and Meththika Vithanage<sup>1,3,4\*</sup>

## Abstract

The increase in antimicrobial resistance (AMR) poses a massive threat to world health, necessitating the urgent development of alternative antimicrobial growth control techniques. Due to their specific physical and chemical properties, nanomaterials, particularly carbon-based nanomaterials, have emerged as attractive candidates for antimicrobial applications, however, reviews are lacking. This comprehensive review aims to bridge the existing knowledge gaps surrounding the mechanism and significance of nanobiochar (NBC) and carbon nanostructures in the field of antimicrobial applications. Notably, NBC, which is derived from biochar, exhibits promising potential as an environmentally-friendly substance with antimicrobial properties. Its strong adsorption capabilities enable the removal and immobilization of pathogens and pollutants from soil and water and also exhibit antimicrobial properties to combat harmful pathogens. In addition to NBC, carbon dots (CDs) and graphene oxide (GO) have also shown excellent antimicrobial properties. These carbon-based nanomaterials find applications in agriculture for phytopathogen control and post-harvest disease management, as well as in medicine for nanotheranostics and in the food industry for extending shelf life as an eco-friendly alternative to chemicals and antibiotics. However, the long-term toxicity of these nanoparticles to humans and the environment needs further investigation, considering the influence of different physiochemical characteristics on antimicrobial properties and nanotoxicity. Therefore, continued exploration in this area will pave the way for future research and safe deployment strategies of carbon-based nanomaterials in combating microbial threats.

## Highlights

- Nanobiochar and carbon dots are promising alternatives to traditional antimicrobial methods.
- Nanobiochar demonstrates strong adsorption and pathogen immobilization properties.
- Carbon dots and graphene oxide show excellent antimicrobial properties.

**Keywords** Carbon dots, Carbon nanotubes, Nanobiochar, Graphene oxide, Antimicrobial activity

Handling editor: Hailong Wang

\*Correspondence:

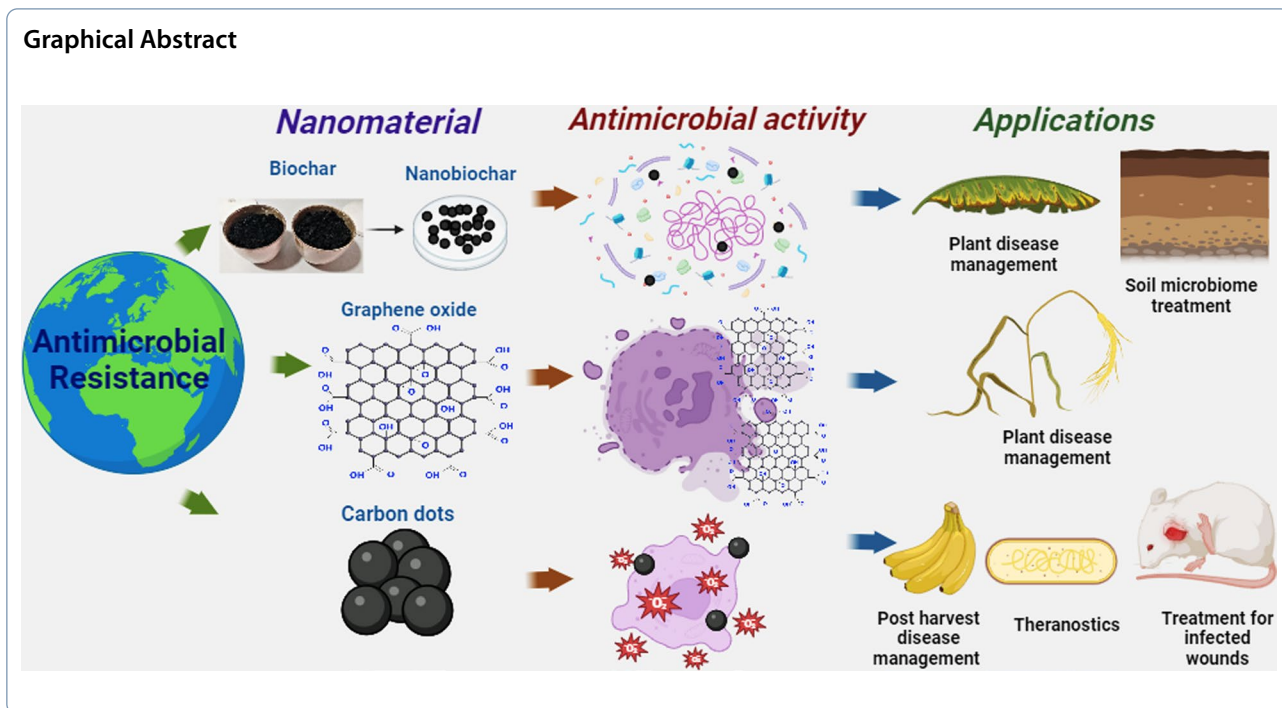
Meththika Vithanage

meththika.vithanage@uwa.edu.au; meththika@sjp.ac.lk

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.



### 1 Introduction

Antimicrobial resistance (AMR) is a critical global health concern that has the potential to become the major cause of death globally in the future decades, with an estimated 4.95 million fatalities attributable to AMR in 2019 and a projected increase to 10 million deaths per year by 2050 (Frei et al. 2023). Antimicrobial resistance poses a threat due to the persistent development of resistant microbes through the overuse of antimicrobial agents in health-care and agriculture, spontaneous microbial evolution, mutations and horizontal gene transfer which allows for the quick acquisition of novel features, such as antibiotic resistance encoded by mobile genetic components, as well as adaptations to changing environmental stresses (Dadgostar 2019). Effective control of microorganisms can be achieved by combining natural antimicrobial macromolecules and their composites, which can reduce the development of pathogen resistance, with physical and chemical agents such as radiation, filtration, temperature, disinfectants, antiseptics, antibiotics and through chemotherapeutic antimicrobial chemicals (Shahrzuzaman et al. 2022). However, challenges remain in achieving optimal efficiency and determining the ideal conditions for decontamination using physical methods (Murmur and Mishra 2018). Furthermore, concerns arise from the improper use of chemical agents, which can lead to the emergence of resistant strains and pose risks to both the environment and human health (Vilaplana et al. 2020). To address these challenges and promote economic

and environmental sustainability in antimicrobial delivery formulations, the utilization of nanomaterials has emerged as a promising strategy (Vurro et al. 2019). Carbon nanomaterials have received recent attention, carbon dots (CDs), carbon nanotubes (CNTs), fullerene (C<sub>60</sub>), graphene oxide (GO), and nanobiochar (NBC), as an option to suppress microbial development (Gurtler et al. 2020).

Carbon nanostructures have received interest as promising alternatives to chemicals and conventional antibiotics in the treatment of multidrug-resistant microorganisms, as they have demonstrated broad-spectrum antimicrobial properties (Al-Jumaili et al. 2017). Carbon dots which were discovered less than 20 years ago have garnered immense popularity among the scientific community due to their low toxicity and biocompatibility (Newman et al. 2021) favouring their application in agriculture and biomedicine. In addition to the low cost involved in their preparation, they could be synthesized from basically any carbon-containing compound, using a variety of strategies, broadly categorized as top-down and bottom-up (Mandal and Das 2022). They exhibit different mechanisms as antimicrobial agents depending on the presence or absence of photoexcitation (Li et al. 2022). Carbon nanotubes, categorized based on the number of layers of graphene cylinders in their structure, are also excellent antimicrobial agents, however, they are limited in use as they pose a risk to human health (Gupta et al. 2019). The use of fullerenes (C<sub>60</sub>) which inhibit

bacterial growth by the generation of reactive oxygen species (ROS) is also discouraged by the same limitation (Azizi-Lalabadi et al. 2020). Graphene oxide is comparatively preferred over CNTs and fullerene due to their low toxicity (Azizi-Lalabadi et al. 2020). However, in comparison to multifunctional uses of nanobiochar, such as environmental cleanup, the development of novel supercapacitors, sensors, and healthcare applications (Ramanayaka et al. 2020a, b), the interplay between NBC and microbe inhibition has received far less attention.

Due to the unique physical and chemical properties, NBC generated from biochar has demonstrated great potential as an effective antimicrobial agent (Lian et al. 2020). However, the underlying mechanism is poorly understood (Pratiwi et al. 2022). Despite the poor understanding of the cytotoxicity of NBC towards microorganisms, previous studies on the interactions of engineered carbonaceous nanoparticles with microbial cells suggest that NBC is potentially harmful due to their strong affinity for heavy metals and other chemicals in the aqueous system (Khare 2021; Chausali et al. 2021). Regardless of expanded studies on NBC and other carbon nanomaterials for antimicrobial applications, the mechanism and significance against different microbial populations remain unclear. Despite the increased focus on studying these materials, there are no reviews discussing the specific mechanisms through which NBC acts against various microbial populations. Therefore, this review addresses current information on the synthesis, antimicrobial mechanisms, applications and knowledge gaps associated with NBC and carbon nanostructures as antimicrobial agents, with the intention of identifying opportunities to conduct further research and effective deployment strategies against different microbial communities.

## 2 Bibliometric analysis on antimicrobial activities of carbon nanomaterials

### 2.1 Method

Using data obtained from the SCOPUS scientific database, a bibliometric analysis of peer-reviewed scientific literature was conducted on the antimicrobial properties of carbon nanomaterials and biochar was conducted. On 25th June, 2023, the titles, abstracts, and keywords of the publications published in the Science Citation Index (SCI) were searched online using the keywords “(“Nanobiochar” OR “carbon dots” OR “carbon nanomaterials”) AND (antimicrobial OR antifungal OR antibacterial OR antiviral)”. Books, editorials, dissertations, and references published in other languages were not included in the search, which only contained English research articles. The search results showed that 639 publications fit these requirements. Based on data from the SCOPUS database, there has been a significant increase in the number of

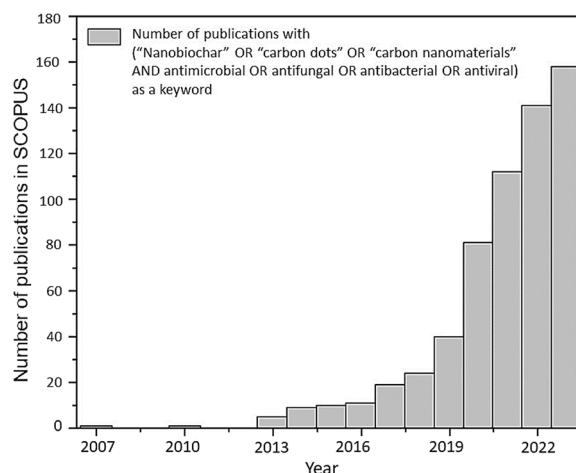
research papers centered around applications of carbon nanomaterials on antimicrobial activities since 2013, as illustrated in Fig. 1.

VOSviewer 1.6.18 software was used for the bibliometric analysis, which uses keyword mapping to determine the essential components of the study. The keyword co-occurrence analysis technique can give useful insights into the relationships between various disciplines of knowledge. In this study, a keyword had to appear at least four times in all of the papers that were retrieved, or four times in total. Seventy five keywords in total satisfied this requirement.

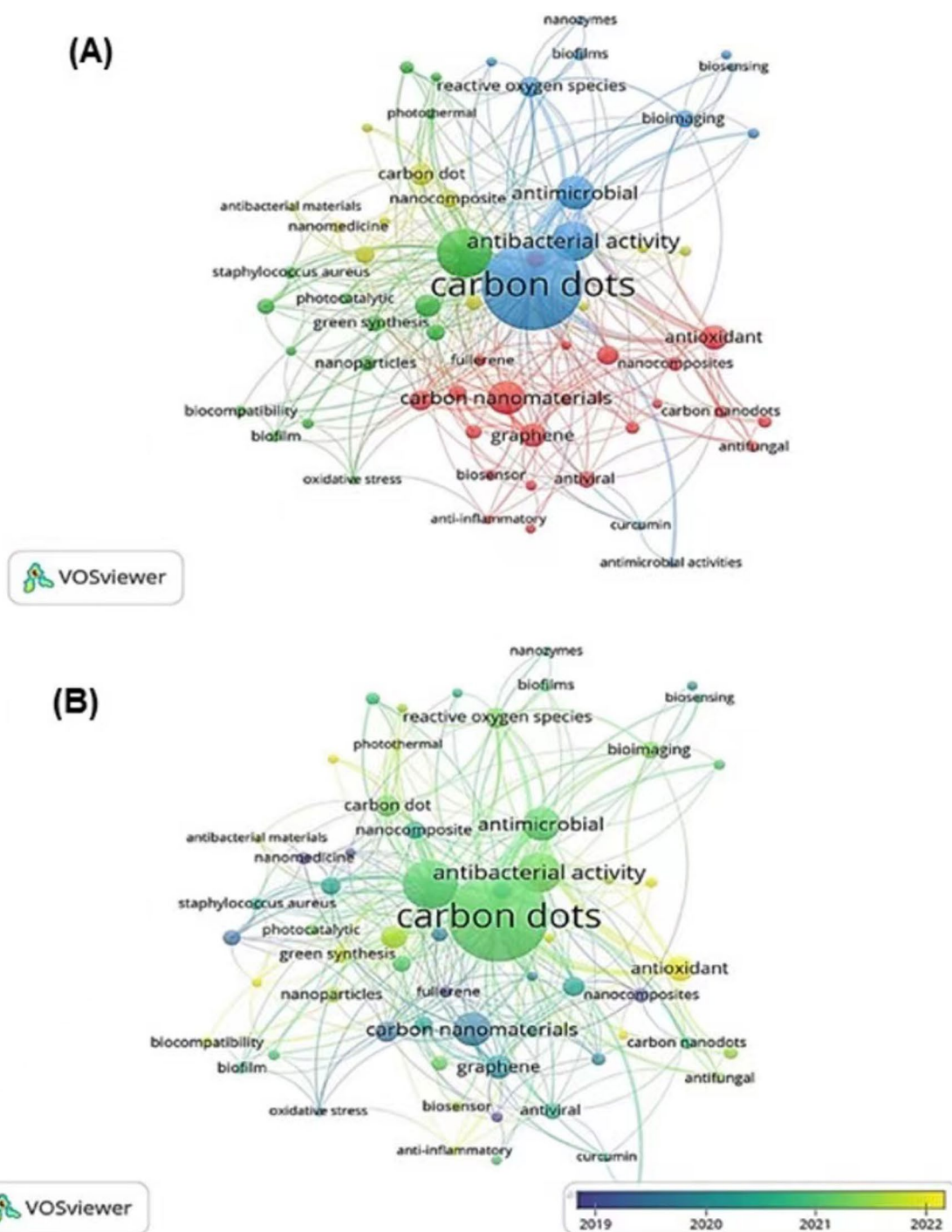
### 2.2 Results

VOSviewer offers three types of visualizations to display bibliometric maps: network visualization, overlay visualization, and density visualization. The network visualization displays the keywords as colored nodes and the relationships between them as edges in a graph. This can help to identify the clusters of related keywords and the strength of their relationships. The overlay visualization overlays different bibliometric maps on top of each other to compare different sets of data, such as the co-occurrence of keywords in different time periods. Furthermore, the size of the nodes and letters used to represent keywords is connected to their frequency of recurrence in publication titles and abstracts. The more often a keyword appears, the greater the size of the letters and nodes.

As per Fig. 2, there is a relationship between node distance and keyword relatedness as evaluated by co-occurrences. In particular, the closeness of two nodes suggests a stronger association between the keywords



**Fig. 1** Number of publications in Scopus with the keywords of “Nanobiochar” OR “carbon dots” OR “carbon nanomaterials” AND antimicrobial OR antifungal OR antibacterial OR antiviral



**Fig. 2** (A) Network visualization and (B) overlay visualization of keywords co-occurrence on antimicrobial activities on nanomaterials

they represent. Figure 2A presents the keyword co-occurrence network, which includes 63 nodes and 331 links. Four clusters of words were found by assessing the keywords based on how frequently they occurred together in the selected publications. Each group was

given a distinct color to represent it. For example, the red color cluster has the highest number of keywords (23), and the most prevalent being “antimicrobial activity”, “antifungal”, “antimicrobial”, “biomaterials”, and “antiviral” which means that there is a close relationship between

them. Figure 2B shows the time distribution of keyword co-occurrence in recent years. Different colors of the node corresponded to the average time of the keyword appearance. If the color of a node approaches yellow, then it indicates that this keyword is in the early stage of development. As shown in Fig. 2B, studies of application of carbon dots on antimicrobial activities have become a trend over the recent past years. The results showed that the term “Carbon dots” was the most commonly used author keyword with 172 occurrences, 52 links to other author keywords, and an average publication year of 2021. The existence of carbon nanoparticles for antibacterial activities up to the year 2019 has attracted a lot of attention among the research that were chosen. As a result, purple nodes appeared corresponding to terms like “carbon nanomaterials” and “nanotechnology”. These keywords are with 34 occurrences, with an average publication year of 2019. With the time researchers are moving their attention away from carbon nanomaterials to carbon dots and nanoparticles for antibacterial activity (starting in 2020). These networks may be used to assess the revolutionary a piece of research is in the subject under consideration.

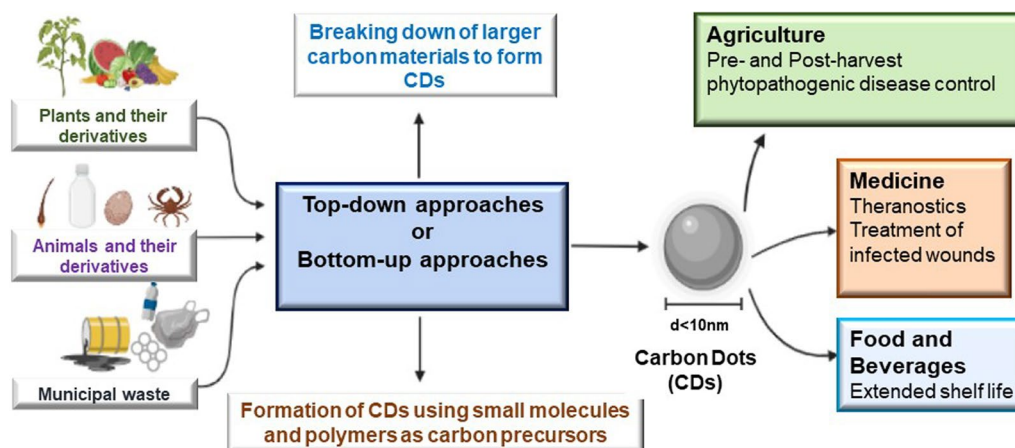
### 3 Carbon nanomaterial-based antimicrobial agents

The antibiotic resistance of microorganisms has implicated the need for more innovative approaches against microbial infections and diseases (Dizaj et al. 2015). Therefore, carbon-based nanomaterials, such as CDs and GO, proffer a promising alternative for the development of effective antimicrobials due to their broad-spectrum antimicrobial properties against pathogens (Al-Jumaili et al. 2017). Although the use of nanoparticles evokes concern about their toxicity and durability,

carbon nanomaterials are favoured over a variety of other nanomaterials, owing to their chemical resistance and the durability of their action (Giraud et al. 2021). However, there is a broad scope for the investigation of their potential toxicity towards humans and the environment at large (Giraud et al. 2021), given that there is an insubstantial focus on their persistence in the human body as well as in varied ecosystems in the long term.

#### 3.1 Carbon dots

Carbon dots also referred to as “carbon quantum dots”, “carbon nanodots” and “carbonized polymer dots” (Lin et al. 2022a, b), are zero-dimensional fluorescent carbon nanoparticles (Dong et al. 2020) with particle sizes less than 10 nm (Pramudita et al. 2022). They have gained a high demand and popularity since their discovery in 2004 due to their low toxicity, low cost, ready availability, high water solubility, dispersibility, photoluminescence, photostability, functional capability and biocompatibility (Newman et al. 2021; Pramudita et al. 2022) which facilitate their potential application as an alternative to conventional antibiotics (Lin et al. 2022a, b). The low cost and the ready availability of CDs could be attributed to the vast array of strategies that could be utilized for synthesising CDs from a variety of biological and chemical precursors rich in carbon content (Newman et al. 2021; Lin et al. 2022a, b). Moreover, the ability to utilize biodegradable sources, such as plants and plant derivatives, animal and animal derivatives and municipal waste, further supports the low-cost, environmentally friendly production of carbon dots on a large scale for potential industrial applications (Lou et al. 2021). Figure 3 summarises the carbon sources that could be utilized for the synthesis of CDs, methods of synthesis and the potential applications based on their antimicrobial properties.



**Fig. 3** The sources used for the carbon dot synthesis and their antimicrobial applications

In addition to different environmental factors such as the light intensity, time of exposure and the concentration of CDs which affect the antimicrobial properties of CDs, several intrinsic factors such as the particle size, precursor used in the synthesis, surface charge and surface functionalization also impact the performance of CDs (Li et al. 2022). When considering the methods for the synthesis of CDs, they may be broadly classified as top-down and bottom-up approaches (Mandal and Das 2022). The former involves techniques such as arc discharge, laser ablation, electrochemical exfoliation and chemical oxidation, where graphite, active-carbon, CNTs, GO and fullerene are broken down to synthesize CDs, whereas the latter involves methods such as hydrothermal, solvothermal, microwave irradiation, direct pyrolysis and sonochemical processes where dehydration, polymerization, aggregation and carbonization of precursor molecules lead to the synthesis of CDs (Mandal and Das 2022; Das et al. 2018). A few of these methods have been compared in Table 1.

Table 2 compares the size, quantum yield, emission peak and the applications of CDs synthesized from plant sources using different methods. Of the methods available, hydrothermal/solvothermal treatment has been a popular choice among researchers for the green synthesis of CDs from natural products, as it is an environmentally-friendly, cheap and simple method (Lin et al. 2021). Nevertheless, even nearly two decades after the discovery of CDs, all the above-mentioned methods suffer from a major limitation, namely the lack of a standard protocol for the synthesis of CDs (Mandal and Das 2022). Hence, the establishment of standard protocols to assist researchers would irrefutably facilitate the reproducibility and consistency in future studies thereby paving way for a much better approach to comparisons of characteristics and yield of CDs and the efficiency of methods utilized, in attempts of determining the most ideal carbon sources and scaling up of laboratory-confined studies for low-cost mass production of CDs.

Moreover, despite extensive characterization of carbon dots synthesized from biomass as well as their application in biological research, there are few reports on the possible synthesis of by-products during CD preparations which may or may not be toxic to humans and the environment at large, since the inclusion of toxic compounds in most biomass resources such as industrial solid waste is plausible. Thus, further research is required to shred more definitive evidence on the suitability and safety of the use of municipal waste for the preparation of CDs.

### 3.2 Graphene oxide

Graphene oxide, derived from the oxidation of graphite by the Hummers' method, followed by the chemical

exfoliation of the formed graphite oxide (Toh et al. 2014) is also considered a promising alternative to antibiotics against multidrug-resistant (MDR) pathogens (Anand et al. 2019). The usage of GO as an antimicrobial is also favoured in biomedical applications due to the solubility of GO in water and aqueous media (Anand et al. 2019), simple, rapid and cheap synthesis and low toxicity for mammalian cells, in addition to the unique thermal and electrical properties (Azizi-Lalabadi et al. 2020).

The antimicrobial properties of GO could be attributed to a variety of factors such as the oxygen-containing functional groups, which render it amphiphilic, the lateral size, morphology and aggregation (Anand et al. 2019). For instance, Wang et al. (2018) reported the differential antimicrobial performance of graphene oxide samples against *E. coli* bacteria (DH5 $\alpha$ ). This study demonstrated how the high number of oxygen-containing functional groups led to better antimicrobial properties of epoxy/graphene oxide composite coatings compared to weak antimicrobial properties of epoxy/reduced graphene oxide coatings (Wang et al. 2018). The increase in antimicrobial performance with the number of oxygen-containing functional groups could be attributed to the oxidative stress induced by the GO sheets. Differences in the bacterial growth inhibition by GO-chitosan (GO-CS) nano-hybrids owing to differences in size and morphology have also been described in literature (Majidi et al. 2019). Here, it was proved that GO-CS nano-fibrillar sample had the highest antibacterial activity against *S. aureus* and *E. coli*, when compared with the GO-CS spherical and GO-CS pristine powder nano-hybrids (Majidi et al. 2019). Thus, one or more factors may synergistically contribute to the antimicrobial properties of GO.

### 4 Antimicrobial mechanisms of carbon-nanomaterials

Different mechanisms are exhibited by CDs as antimicrobial agents (Li et al. 2022). In the absence of photoexcitation (Fig. 4A), these mechanisms involve adsorption to bacterial and fungal cell walls via diffusion and electrostatic interactions leading to alteration of the cell wall and membrane permeability (Li et al. 2022). The penetration of CDs through bacterial and fungal cell walls and membranes leads to cellular cytoplasm leakage and the DNA and RNA binding by CDs results in the destruction of the nucleic acid structures, thereby inhibiting the growth of bacteria (Li et al. 2022). However, in the presence of photo excitation (Fig. 4B), these CDs generate ROS, which induce oxidative damage to bacterial cells and alter the expression of essential genes, resulting in the death of bacterial cells (Li et al. 2022). It has also been recorded that the antibacterial activity is enhanced when visible light has been used to activate CDs prepared

**Table 1** Comparison of a few common synthesis methods for Carbon Dots

Methods	Processes	Benefits	Limitations	References
Arc discharge	Purification of single-walled carbon nanotubes by nitric acid oxidation of arc ash	Have small particle sizes Large oxygen contents Emit fluorescence without surface modification	Have complex compositions The impurities present are difficult to be removed and extracted	Wang et al. (2017)
Laser ablation	Irradiation of carbon targets immersed in water using direct UV-pulsed laser	Simple, clean and controllable method High thermal/chemical stability of the Carbon dots	The sizes are heterogeneous Low yield Low utilisation efficiency	Castro et al. (2016); Wang et al. (2017); Das et al. (2018)
Chemical oxidation	Oxidation of the carbon precursor with a strong oxidant	Simple operation Low cytotoxicity High biocompatibility Large-scale production can be done using low-cost carbon raw materials	Strong oxidants are required	Wang et al. (2017)
Hydrothermal/ solvothermal methods	Autoclaving of an aqueous solution of the carbon precursor at a high temperature	Simple, low-cost method Surface modification is not required Mass production is possible High dispersibility of the Carbon Dots in water and other common solvents	The sizes of the Carbon Dots are not uniform Presence of impurities	Wang et al. (2017)
Microwave synthesis	Carbonization of carbon precursors in a short period by microwave heating	Simple and rapid process Efficient process Strong fluorescence of the Carbon Dots Excellent biocompatibility	Low yield The particle sizes are not uniform Purification is difficult	Wang et al. (2017); Das et al. (2018)
Direct pyrolysis	Carbonization of carbon precursors at a high temperature	Simple and repeatable process High photoluminescence quantum yield	An alkali or strong acid of high concentration is required Purification is difficult	Wang et al. (2017)
Sonochemical synthesis	Generation of alternating high- and low-pressure waves in a liquid medium using ultrasonic waves	Rapid, reproducible and scalable green process Excellent biocompatibility and fluorescent intensity of the Carbon Dots	Low stability of the bare Carbon Dots Low fluorescence Poor quantum yield	Dehvari et al. (2019); Kumar et al. (2020)

**Table 2** Synthesis of carbon dots from plant sources

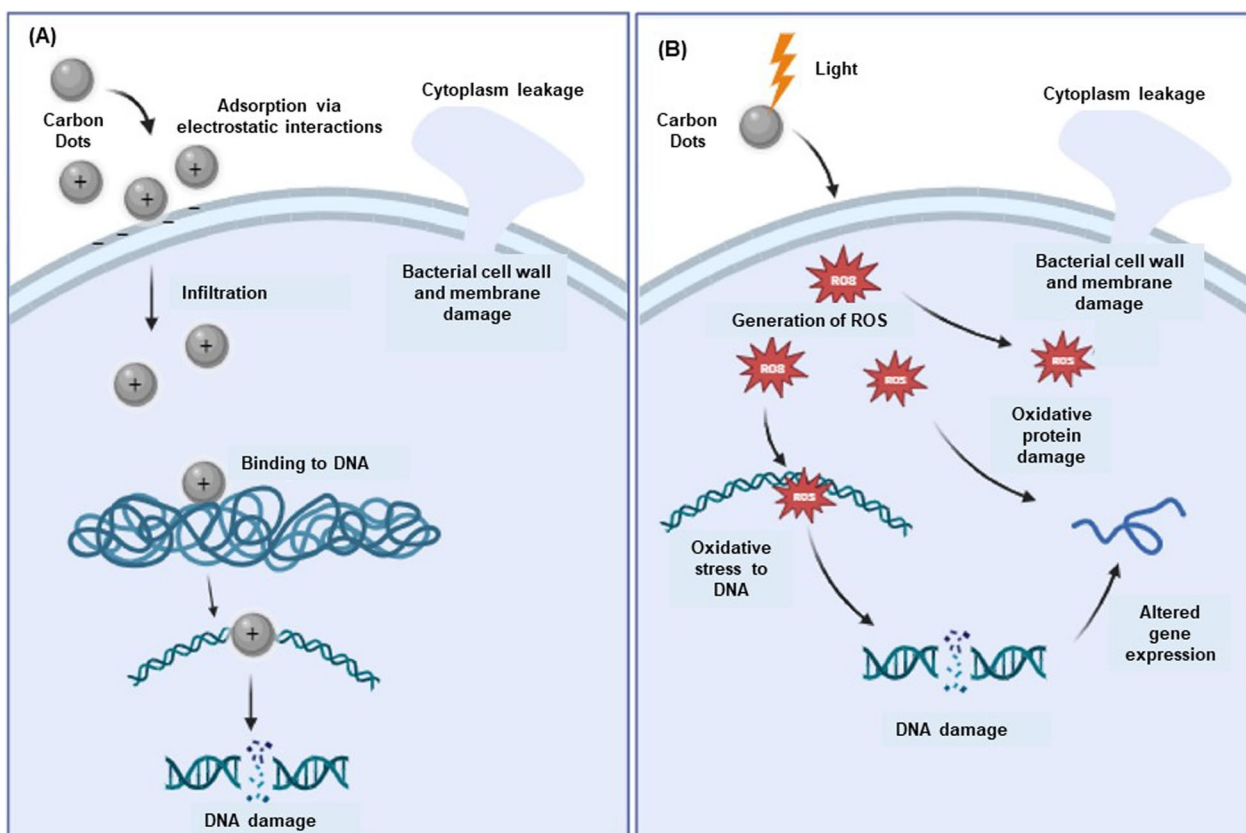
Carbon sources	Methods	Sizes (nm)	Quantum Yields (%)	Emissions (nm)	Applications	References
Citric acid	Hydrothermal	3.0–6.0	48.3 ± 5.3	459	Determination of morin in human urine samples	Li et al. (2017)
Walnut oil	Hydrothermal	12.3 ± 2.7	14.5	430	Cytotoxic and apoptotic potential on prostate and breast cancer cells (potential for cancer chemotherapy)	Arkan et al. (2018)
Ginger juice	Hydrothermal	8.2 ± 0.6	13.4		Inhibition of human hepatocellular carcinoma cells (HepG2) (potential for the treatment of liver cancer)	Li et al. (2014)
Rose-heart radish	Hydrothermal	1.2–6.0	13.6	420	Environmental Fe <sup>3+</sup> detection; Cell imaging in biomedical fields	Liu et al. (2017)
Peppermint ( <i>Mentha piperita</i> ) essential oil	Bottom-up green pyrolysis	NI	NI	310	NI	Rimal and Srivastava (2022)
Polyphenol-enriched pomegranate peel extract	Hydrothermal	6.1 ± 1.2	20.51		Antibacterial study; Cytotoxicity study; Antioxidant activity assay	Sattariazar et al. (2023)
Lemon and onion juices	Microwave-assisted carbonization	4.23–8.22	23.6	425	Determination of riboflavin in multivitamin/mineral supplements	Monte-Filho et al. (2019)
Essential oils Clove ( <i>Eugenia caryophyllata</i> ), Basil ( <i>Ocimum basilicum</i> ), Turmeric ( <i>Curcuma longa</i> ) and Cardamom ( <i>Elettaria cardamomum</i> )	Bottom-up green pyrolysis	Clove = 1.744–8.844; Basil = 1.822–3.516; Turmeric = 1.755–4.62; Cardamom = 1.012–11.313	NI	NI	Antifungal effect against <i>Aspergillus</i> , <i>Penicillium</i> and <i>Fusarium</i> sp.; Growth retarding effect on <i>Spinach</i> ; Antiproliferative activities in MCF-7 (human breast carcinoma) cell lines	Rimal and Srivastava (2022)
Saffron	Hydrothermal	3.5–7.5	23.6	485	Cell imaging and sensing of pilocaine in biological samples	Ensafi et al. (2017)
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Hydrothermal	16.13	NI	422	Food storage (Antibacterial and antioxidant activity); Fingerprint detection	Eskalen et al. (2020)



**Table 2** (continued)

Carbon sources	Methods	Sizes (nm)	Quantum Yields (%)	Emissions (nm)	Applications	References
Onion	Hydrothermal	NI	NI	NI	Bacteriostatic agent for aquatic products	Lin et al. (2022b)

NI not indicated



**Fig. 4** Antimicrobial mechanisms of Carbon Dots in the (A) absence and (B) presence of photoexcitation

with antibiotics as a precursor (Sidhu et al. 2017). Hence, using antibiotics or antibacterial compounds as the precursor presents a more efficient alternative to tackling MDR bacteria (Sidhu et al. 2017).

The antimicrobial activity of GO is demonstrated through the generation of ROS (Fig. 5) (Azizi-Lalabadi et al. 2020), resulting in oxidative stress, DNA fragmentation and eventual cell death of microorganisms (Anand et al. 2019). It has also been reported that GO is involved in mechanisms such as cell membrane damage by direct cutting by the sharp edges of the GO nanosheets, mechanical wrapping on bacterial cells leading to cell lysis and extraction of phospholipids from the membrane (Anand et al. 2019). Thus, the synergistic effect of these mechanisms may prove GO to be more effective as an antimicrobial agent compared to other carbon-based nanomaterials such as CNTs and fullerene, which suffer the limitations of insolubility and potential toxicity to humans.

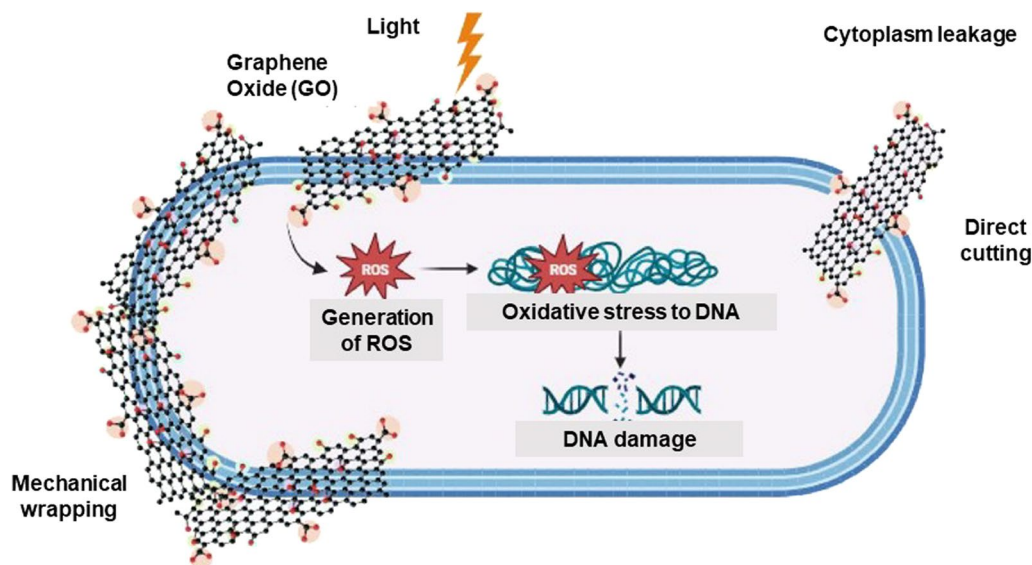
## 5 Nanobiochar as an antimicrobial agent

Nanobiochar, characterized by particle sizes less than 100 nm, exhibits unique physicochemical properties that distinguish it from bulk biochar (Liu et al. 2018). These

properties, such as a larger surface area, graphitic nature, highly negative zeta-potential, and diverse crystalline forms, contribute to its enhanced stability (Oleszczuk et al. 2016) and temperature-dependent dispersibility compared to bulk biochar (Ramanayaka et al. 2020a, b). The antimicrobial activity of NBC is influenced by its physicochemical properties, including size, shape, chemical modification, coating, and combination with other nanoparticles and solvents (Pratiwi et al. 2022). These factors can significantly impact the interaction between NBC and microbial cells, ultimately affecting its antimicrobial effectiveness. However, it is important to note that there is currently a lack of studies exclusively focusing on the antimicrobial activity of NBC. Therefore, the exact antimicrobial mechanism of NBC remains poorly understood. Further research is needed to investigate and elucidate the specific mechanisms through which NBC interacts with microbial communities and exerts its antimicrobial effects.

### 5.1 Synthesis of nanobiochar

The synthesis of NBC involves the initial production of biochar, which can be achieved through various processes such as pyrolysis, hydrothermal carbonization,



**Fig. 5** Antimicrobial mechanisms of Graphene Oxide

gasification, torrefaction, flash carbonation, microwave pyrolysis, mechanochemical technology, and engineered biochar (Jeyasubramanian et al. 2021; Yaashikaa et al. 2020; Rajapaksha et al. 2016; Zhou et al. 2021). Each of these processes has its own set of conditions and parameters that influence the characteristics and the yield of biochar. Factors such as the type of feedstock, temperature, heating rate, residence duration, and pressure points play crucial roles in determining the properties of biochar (Naghdi et al. 2017).

The top-down approaches are widely utilized as the predominant methods for NBC production (Ramanayaka et al. 2020a, b). These methods allow for control over the size, shape, and surface properties of NBC (Naghdi et al. 2017). Ball-milling is a popular mechanical procedure that can be used to synthesize NBC. It applies mechanical force to a material using a high-energy ball mill to break it down to the nanoscale level while preserving the crystal structure. This method allows for the production of NBC with controlled particle size and shape (Ramanayaka et al. 2020a, b). Vibration disc milling is another option that is preferred over ball milling for generating NBC particles of consistent size and shape. However, double-disc milling is not commonly used for NBC preparation due to the high operational cost associated with this method (Chausali et al. 2021). As reported by Jiang et al. (2023), ultrasonication, microwave pyrolysis, centrifugation technology, digestion using concentrated nitric and sulfuric acids stand out as other effective techniques for the production of nanobiochar.

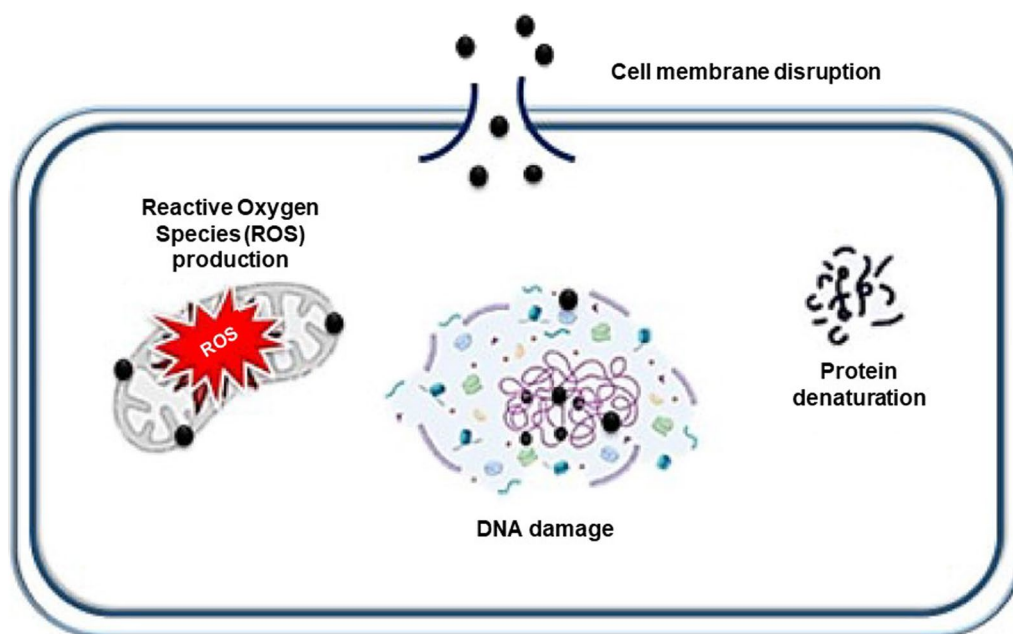
Overall, the synthesis of NBC involves a combination of different processes and techniques, depending on the

desired characteristics and properties of the nanoparticles. The choice of method can significantly impact the size, shape, and surface properties of NBC particles, which, in turn, can influence their antimicrobial activity and other applications. Understanding and optimizing the synthesis methods are essential for producing NBC with desired properties for various applications.

## 5.2 Action of nanobiochar against microbial communities

The interactions between NBC and microbial communities can have both beneficial and adverse effects. Nanobiochar, although it has the potential to enhance microbial activity and promote bioremediation, can also pose risks to microbial populations by generating persistent free radicals (PFRs) and reactive oxygen species (ROS), which may impact microbial abundance and diversity in soil and water environments (Fang et al. 2015). The presence of NBC can result in the release of ions, leading to cellular uptake, DNA damage, accumulation of ROS, and subsequent intracellular damage in microorganisms, potentially affecting their physiological processes and overall cellular health (Esmailzadeh et al. 2016).

However, NBC also demonstrates antibacterial properties that can be beneficial in certain applications. Nanobiochar can be coated with extracellular polymeric substance in aqueous environments, which can affect its dispersion and antimicrobial action. This coating process may lead to the decomposition of NBC and the release of ions, causing antimicrobial effects (Esmailzadeh et al. 2016). For example, a study by Naghdi et al. (2017) demonstrated the antibacterial activity of a Chitosan-Nanobiochar composite encapsulating laccase, which effectively



**Fig. 6** Schematic representation of toxicology effect of nanobiochar on bacterial cell

degraded pollutants by immobilized laccase and exhibited antibacterial activity against *Bacillus subtilis*. As depicted in Fig. 6, the entry of NBC into the cytoplasm of microorganisms has been observed through two different mechanisms: membrane breakdown or uptake through cell mediatory organelles (Pratiwi et al. 2022). This internalization of NBC particles can potentially have direct effects on microbial cells, affecting their physiological processes and intracellular structures. Furthermore, NBC has been shown to adsorb and fragment extracellular DNA, leading to significant inhibition of DNA replication. Hydroxyl radicals generated from PFRs on NBC are primarily responsible for the damage to DNA, while the direct interaction between nonradical reactive sites and PFRs on NBC also contributes to DNA degradation (Lian et al. 2020).

Overall, while NBC holds promise in enhancing microbial activity and promoting bioremediation, its effects on different microbial cells and communities are still not fully understood. Further research is needed to elucidate the precise mechanisms by which NBC interacts with microbial cells and the potential risks and benefits associated with the antimicrobial activity.

## 6 Applications of nanobiochar and carbon nanomaterials based on antimicrobial activities

### 6.1 Agriculture

Agriculture could be considered a significant sector to benefit from using nanomaterials as it involves ensuring sustainable food production and food security in the face

of rapid population growth and climatic changes (Omran and Baek 2022). The use of nanotechnology in agriculture could be focused on the control of phytopathogens and promoting plant growth (Mittal et al. 2019). The application of nanobiochar and other carbon nanomaterials in agriculture to effectively overcome the limitations of conventional measures for the control of phytopathogens and thereby, control post-harvest losses of fruits and vegetables in particular. However, it must be understood that the use of nanomaterials may also be presented with limitations as well. Research has demonstrated that nanobiochar have a direct positive impact on the growth of rhizosphere microorganisms. It has been observed that soil samples treated with nanobiochar exhibited higher microbial biomass and a greater diversity of soil microorganisms compared to untreated soil samples (Zhang et al. 2022). Hence, additional research is necessary to accurately ascertain the antimicrobial properties of nanobiochar in relation to the inhibition of phytopathogen development (Table 3).

#### 6.1.1 Conventional measures for the control of phytopathogens

Significant crop losses due to post-harvest diseases caused by phytopathogens have the potential to even lead to starvation and death (Omran and Baek 2022). Hence, from ancient times traditional agricultural practices have been followed to control post-harvest losses. Plant diseases can be managed via chemicals, resistant cultivars,

**Table 3** Antimicrobial applications and optimal conditions for the antimicrobial performance of carbon nanomaterials

Nanomaterial types	Applications	Optimal Conditions	Performances	References
Carbon Dots	Control of phytopathogenic fungi	Temperature = 37 °C Time = 48 h	Inhibition of growth of <i>Rhizoctonia solani</i> at a concentration of 300 µg mL <sup>-1</sup>	Li et al. (2018)
		Temperature = 37 °C Time = 48 h	Inhibition of growth of <i>Pyricularia grisea</i> at a concentration of 300 µg mL <sup>-1</sup>	
	Control of phytopathogenic bacteria	Time = 5 – 6 h	Complete inhibition of growth of <i>Pectobacterium carotovorum</i> Ecc7, <i>Agrobacterium tumefaciens</i> EHA101, <i>A. rhizogenes</i> K599 and <i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000 at a concentration of 19 mg mL <sup>-1</sup>	Pandey et al. (2021)
		Temperature = 37 °C Time = 48 h	Inhibition of growth of <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> at a concentration of 100 µg mL <sup>-1</sup>	Li et al. (2018)
Antimicrobial protective nanopaper	Control of pathogenic bacteria	Temperature = 37 ± 1 °C Time = 24 h	Antibacterial activity towards foodborne pathogens; <i>Escherichia coli</i> and <i>Listeria monocytogenes</i> , at a concentration of 500 mg mL <sup>-1</sup>	Kousheh et al. (2020)
		Impregnation temperature = 30 °C Impregnation time = 14 h Temperature = 37 °C Incubation time = 24 h	Antimicrobial activity on <i>Listeria monocytogenes</i> at a concentration of 530 g L <sup>-1</sup>	Salimi et al. (2021)
	Antibacterial photodynamic therapy for wound healing in mice	Dose of LED light = 60 J cm <sup>-2</sup> Wavelength of LED light = 450 nm Exposure time = 28 min	Reduction of 10 <sup>4</sup> log of <i>Staphylococcus aureus</i> and complete healing of skin lesions in Swiss mice at a concentration of 6.9 mg mL <sup>-1</sup>	Romero et al. (2021)
Graphene Oxide	Bactericidal agent against multidrug resistant hospital superbugs	Temperature = 37 °C	Antibacterial activity against <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus mirabilis</i> , <i>Serratia marcescens</i> and <i>Staphylococcus aureus</i> at a concentration of 1 µg µL <sup>-1</sup>	Aunkor et al. (2020)
		Temperature = 37 °C Time = 24 h	Antibacterial activity against <i>Bacillus subtilis</i> , <i>Staphylococcus epidermidis</i> , <i>Pseudomonas aeruginosa</i> and <i>Enterobacter aerogenes</i> at a concentration of 100 µg mL <sup>-1</sup>	Gupta et al. (2015)
	Nanocomposite film for food packaging	Temperature = 37 °C Time = 24 h	Antibacterial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> at a GO concentration of 1% w/w	Aifat et al. (2018)

biological controls, cultural practices, physical treatments and nutrition management (Miah et al. 2017; Pandit et al. 2022) as depicted in Fig. 7.

Chemical pesticides are essential to modern agricultural efforts to improve food safety and increase crop output and quality both preventively and therapeutically (Raymaekers et al. 2020). However, the broad use of chemicals such as pesticides and fungicides is implicated in several health concerns such as cancer in humans and environmental hazards due to their ecotoxicity and bioaccumulation (Pandit et al. 2022). It has also led to the emergence of resistant strains of pathogens, thereby reducing the effectiveness of the treatment (Ruffo et al. 2019). The disease-resistant nature of genetically modified crops makes them a cost-effective option that can lead to higher yields without the need for costly chemical inputs (Pandit et al. 2022). There seems to benefit from other breeding techniques including gene rotation, gene pyramiding, and multiline varieties for managing resistance (Pandit et al. 2022). Many agonistic and antagonistic interactions between plants and microorganisms in the

rhizosphere and phyllosphere are necessary for biological control as it is extremely specific, economically feasible and safe for the environment (Mishra et al. 2015). When considering biological control measures, microbial biocontrol agents have become ineffective with the emergence of new serotypes due to the high specificity of the biocontrol agents (Rajwade et al. 2020). Agronomic strategies such as field hygiene, crop rotation and regulatory measures including eradication and quarantine, have been undertaken in addition to biological, physical and chemical measures for controlling pathogens (Omran and Baek 2022). To minimize the spread of pests and diseases, crop rotation, thorough tillage, weeding and modifying sowing and harvesting times should be used as cultural practices (Gupta et al. 2017). This is a crucial preventative measure, but it will not completely eradicate the condition (Miah et al. 2017). Physical treatments, such as irradiations, ozone treatment and the use of electrolyzed water, may also suffer the disadvantages of high initial investment, and high operation and maintenance costs (Deng et al. 2020). To replenish the soil nutrient supplies

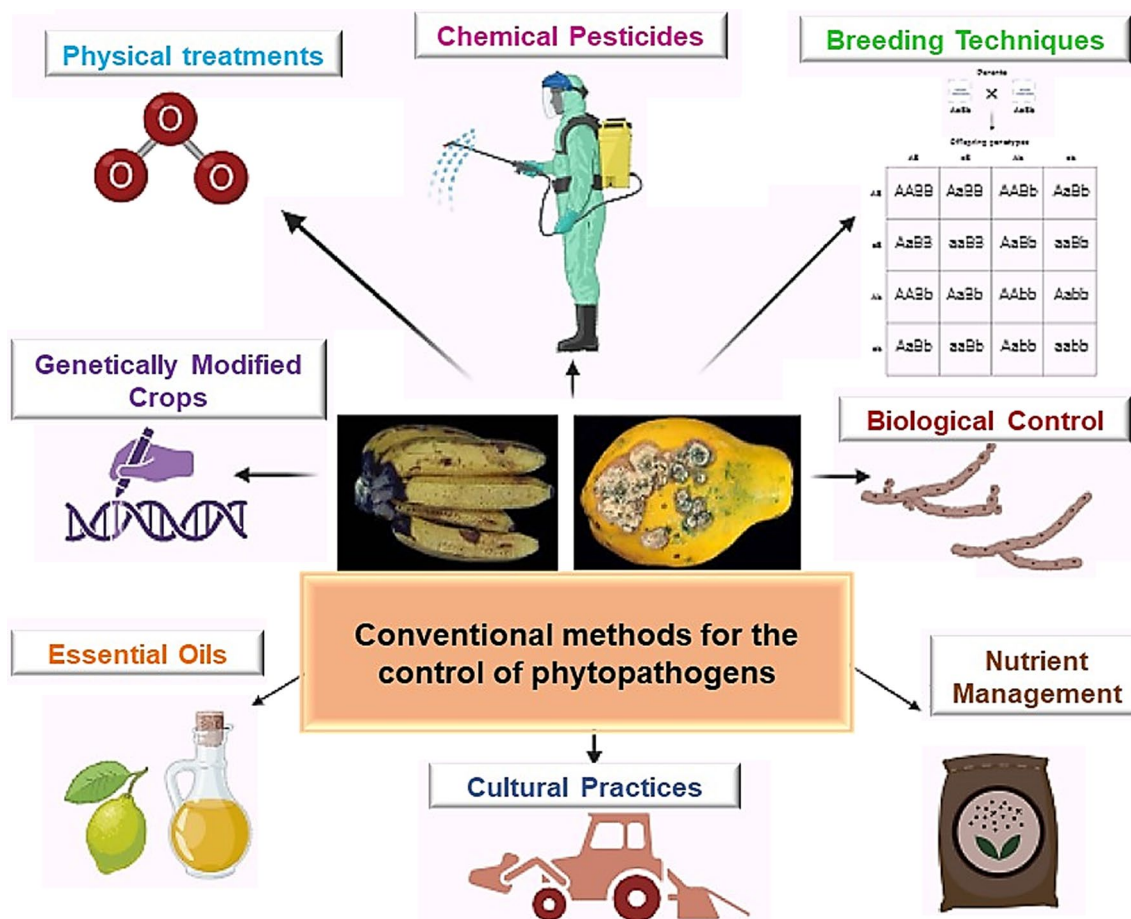


Fig. 7 Conventional measures to control phytopathogens

depleted after crop removal, the only option available is to introduce external sources of nutrients (Gupta and Gupta 2016). The nutrients available to a plant have a significant impact on its histology or morphology, as well as the ability of any infection to persist within the host (Gupta et al. 2017). Therefore, by providing crops with a sufficient and balanced mineral diet, illnesses can spread at a slower rate (Gupta et al. 2017). Furthermore, the use of essential oils, which is a safer alternative that does not lead to resistance development by pathogens, has a limited application due to their volatility and susceptibility to degradation (Nair et al. 2022). Hence, effectively managing diseases caused by phytopathogens requires an alternative that overcomes these limitations.

### 6.1.2 Post-harvest disease management

Pathogen infestation in crops, caused by various types of pathogens such as viroids, viruses, bacteria, fungi, oomycetes, and nematodes, results in substantial crop losses each year (Pandit et al. 2022). A study by Aftab et al. (2022) demonstrated that a combination of rice straw biochar nanoparticles (RSBNPs) and fly ash nanoparticles (FNPs) effectively inhibited bacterial leaf spot of pepper caused by *Xanthomonas campestris* pv. *vesicatoria*. X-ray diffractometry analysis highlighted the unique composition of RSBNPs and FNPs, which likely contributed to enhancing plant defense mechanisms against the invading *X. campestris* pv. *vesicatoria*, while RSBNPs showed a growth inhibition rate of 51.2% and FNPs exhibited an inhibition rate of 42.4% compared to the control. The activity of these NBC materials against the pathogen can be attributed to several factors. Firstly, the small particle size of the RSBNPs and FNPs provides a larger surface area for interactions with the pathogen, increasing the chances of physical contact and disruption (Pratiwi et al. 2022) of its growth and reproduction. Additionally, the unique properties of the NBC materials, such as their high surface reactivity, porous structure (Ramanayaka et al. 2020a, b), and ability to absorb and retain water and nutrients (Razzaghi et al. 2020), contribute to creating an unfavorable environment for the pathogen. These materials can potentially limit the availability of essential resources which are required for the survival of the pathogen (Kościak et al. 2021), impair mobility, and hinder the ability to colonize and infect plant tissues. Therefore, these materials hold promise in addressing problematic soils and have the potential to enhance the growth and yield of crops.

Carbon nanomaterials have also been popularly accepted as a fitting candidate for alternative and better means of controlling phytopathogens owing to their intrinsic properties which differ greatly from those of bulk material (Roberto et al. 2019). When considering the

use of carbon dots, there are only a few studies focused on their use in agriculture, thereby implying a wide scope for research in the future. Nevertheless, many findings have implied the potential of CDs as broad-spectrum antimicrobial agents (Baruah and Sahu 2022). Degradable CDs have been synthesized from Vitamin C using a one-step electrochemical method (Li et al. 2018). They have demonstrated broad-spectrum antimicrobial properties through the antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus* sp. WL-6, *Escherichia coli* and ampicillin-resistant *Escherichia coli* at a concentration of 100  $\mu\text{g mL}^{-1}$  and the antifungal activity against *Rhizoctonia solani* and *Pyricularia grisea* at a concentration of 300  $\mu\text{g mL}^{-1}$  (Li et al. 2018). Moreover, Pandey et al. (2021) reported the antibacterial activity of CDs synthesized using citric acid as a carbon source and  $\beta$ -alanine as a surface passivator. These CDs have exhibited an enhanced antibacterial activity against a group of Gram-negative bacteria, *Escherichia coli*, *Pectobacterium carotovorum*, *Agrobacterium tumefaciens*, *A. rhizogenes*, *Pseudomonas syringae* pv. *tomato* and *Salmonella enterica* subsp. *enterica* serovar *typhimurium* at a concentration of 19  $\text{mg mL}^{-1}$  compared to that of a solution of citric acid and  $\beta$ -alanine (Pandey et al. 2021). Although the antimicrobial property was demonstrated to be light-dependent, the exact mechanisms involved have not been precisely determined (Pandey et al. 2021). Antifungal activity of CDs in the control of phytopathogenic diseases has been however explored in depth have reported the synthesis of CDs with 2-methoxy-1,4-naphthoquinone (MNQ), an antifungal agent with poor water solubility, isolated from *Impatiens balsamina* Linn, which is a Chinese medicinal plant, as a carbon source (Chen et al. 2022). These MNQ-based CDs have been found to have a MIC of 2.8  $\mu\text{g mL}^{-1}$  on *Penicillium italicum*, a citrus blue mould which causes huge post-harvest losses of citrus fruits (Chen et al. 2022). In this study, transcriptomics has been integrated with metabolomics to reveal the differential expression of genes and differentially accumulated metabolites upon MNQ-based CD treatment (Chen et al. 2022). Their findings have been supported by transmission electron microscopic (TEM) observations and reverse transcription-quantitative PCR (RTq-PCR) analysis (Chen et al. 2022). Thus far, several studies have confirmed the effectiveness of CDs in controlling phytopathogenic bacteria and fungi.

Moreover, GO has also exhibited broad-spectrum antimicrobial activity against both bacteria such as *Pseudomonas syringae* and *Xanthomonas campestris* pv. *undulosa* and fungi such as *Fusarium graminearum* and *Foxysporum*, leading to their loss of mass and inhibition of spore germination, respectively, thereby indicating a potential to be adopted as a highly efficient antimicrobial

agent for the control of diseases such as *Fusarium* head blight, bacterial leaf streak and bacterial leaf blight in wheat crops (Chen et al. 2013).

### 6.2 Treatment of soil microbiome

Rashid et al. (2023) stated that higher concentrations of NBC derived from farmyard manure biochar resulted in a significant decrease in microbial biomass, potentially leading to toxicity for soil microbes and their associated ecosystem services. This toxicity could be attributed to the unique characteristics of NBC, such as its smaller size, high surface area, and negative surface charge (Shafiq et al. 2023), which can have varying effects on soil microbes and their processes depending on the concentration applied. Therefore, caution should be exercised when recommending the use of NBC in agroecosystems to improve soil quality and crop production, as it could have adverse effects on soil microorganisms similar to the observed production of ROS and resulting oxidative stress and cell damage in algal cells by Zhao et al. (2017).

### 6.3 Theranostics

The potential of CDs as theranostic agents has been evaluated in many studies where CDs have demonstrated antimicrobial activity through labelling and eradication of bacterial cells. Yang et al. (2019a, b) synthesized quaternized CDs using glycerol and quaternary ammonium-carrying organosilane (dimethyloctadecyl [3-(trimethoxysilyl)propyl] ammonium chloride, abbreviated as Si-QAC) through a one-step solvothermal method and these CDs exhibited polarity-sensitive, multicolour fluorescence emission, thereby revealing an increase in fluorescence emission upon contact with Gram-positive *Staphylococcus aureus*, which was absent upon contact with Gram-negative *E. coli* (Yang et al. 2019a, b). They were also shown to inhibit bacterial growth of *S. aureus* at a minimum inhibitory concentration (MIC) of 4  $\mu\text{g mL}^{-1}$  whereas a high MIC of 100  $\mu\text{g mL}^{-1}$  was required to inhibit the growth of *E. coli*. This could be attributed to the surface structure of the CDs and the bacterial cell surfaces and this selective fluorescence emission and bacterial inactivation of Gram-positive bacteria indicate the potential application of CDs in bacterial infection-oriented theranostics. Other carbon nanomaterials discussed in this review have also been found to be useful in cancer nanotheranostics as drug delivery systems, platforms for biosensing and bioimaging, photosensitizers and probes for live cell imaging in the near-infrared region, thereby preventing side effects of conventional chemotherapy due to inappropriate drug distribution (Kościak et al. 2021). Haider et al. (2023)

reported the surface functionalization of graphene oxide quantum dots (QD) with a peptide having a high affinity to placenta-specific protein-1 (PLAC-1) which is overexpressed in colorectal cancer (Haider et al. 2023). This study demonstrated the ability of the peptide-functionalized QD to favour the uptake of malignant cells, in addition to increased cell toxicity and reduced metastatic potential of colorectal cancer cell lines (Haider et al. 2023).

### 6.4 Food storage

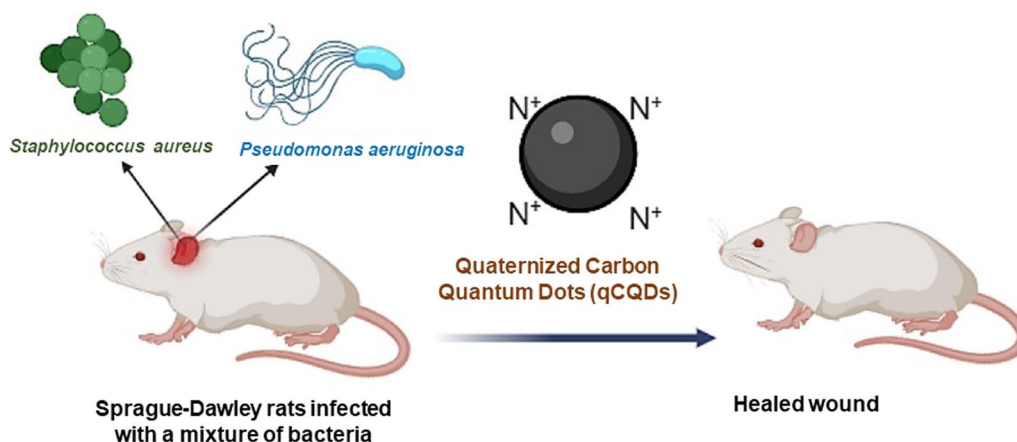
Blue fluorescent CDs hydrothermally synthesized using rosemary (*Rosmarinus officinalis* L.) leaves as a carbon source have exhibited their potential in food storage when they were used as a coating on physiologically mature but unripe bananas, in combination with polyvinyl alcohol, a biodegradable synthetic polymer (Eskalen et al. 2021). This study indicates an example of synthesizing edible food coatings for the preservation of perishables as a post-harvest disease control measure. Similarly, onion CDs have demonstrated their potential to be applied on aquatic products as a means of preservation of seafood (Lin et al. 2022a, b). This is due to their antibacterial activity against *Pseudomonas fragi* at a MIC of 2  $\text{mg mL}^{-1}$  and the extended shelf life of Atlantic mackerel (*Scomber scombrus*) at 4 °C (Lin et al. 2022a, b).

### 6.5 Treatment of biofilms and infected wounds

Biofilms consist of mixed populations of Gram-positive and Gram-negative bacteria and quaternized carbon quantum dots synthesized by a simple green 'one-pot' method with dimethyl diallyl ammonium chloride and glucose as precursors have been proved to be effective at the treatment of wounds infected with mixed bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in Sprague–Dawley rats (Zhao et al. 2022) as shown in Fig. 8. The findings of this experiment could potentially be extrapolated for the treatment of wounds in humans, especially in immunocompromised individuals, by a proper understanding of the underlying mechanism.

Moreover, most studies are involved in biomedical applications where they emphasize the antibacterial activity of CDs hydrothermally synthesized with plant parts as the carbon source, against human pathogenic bacteria. One such instance is the antibacterial activity of CDs synthesized from turmeric (*Curcuma longa*) leaves against *Escherichia coli* and *Staphylococcus aureus* at a minimum inhibitory concentration (MIC) of 0.25  $\text{mg mL}^{-1}$  due to the generation of ROS (Saravanan et al. 2021).





**Fig. 8** Quaternized carbon quantum dots as a treatment of wounds in Sprague-Dawleys rats

## 7 Potential risks, challenges and future perspectives

Despite the claims of the low toxicity of CDs compared to other carbon nanomaterials (Newman et al. 2021), the mode of synthesis and physicochemical characteristics such as size, surface charge, chemical composition and aggregation in culture medium were found to influence CD toxicity when the loss of cell viability of THP-1-derived macrophages, a model of human macrophages, was assessed (Fan et al. 2019). Hence, it was understood from this study that the prediction of the safety of CDs is not an easy task as several factors affect the nanotoxicity of CDs. Other carbon-based nanomaterials such as CNTs and fullerenes have also raised concerns over human health, thereby demeriting their wide application in the absence of functionalization or surface modification (Azizi-Lalabadi et al. 2020).

Apart from carbon nanostructures, the limited availability of studies assessing the toxicity of NBC on different plant species, mammalian cell systems, and soil microbial communities, it is crucial to address their potential biological and environmental toxicity (Chausali et al. 2021). The high dispersion of NBC in natural waters, along with their associated contaminants, has the potential to pose exposure risks to aquatic organisms, resembling those associated with engineered carbonaceous nanomaterials (Liu et al. 2018). Nanobiocar particles have the potential to become airborne during their manufacturing, handling, and application processes, which may pose risks such as respiratory problems and eye irritation upon contact. While initial examinations of their impact on the respiratory system suggest a relatively lower risk to human health (Dong et al. 2019). However, additional research is required to assess the potential health risks associated with NBC and its impact on human health compared to CDs. In

order to obtain a comprehensive understanding of NBC toxicity towards microbes, further analysis is needed to investigate the impact on microbial growth, proteomic levels, and metabolic responses (Pratiwi et al. 2022). To address the major drawback of low yield in large-scale production of NBC and ensure cost-effective production, research should prioritize optimizing production methods to achieve higher yield with desired properties (Khare 2021).

Despite the acknowledgement of CDs as a better alternative to conventional antibiotics for tackling multidrug-resistant microorganisms, there is still great confusion among the scientific community on the proper nomenclature of CDs, as they attempt to address them by names which reflect their precursor molecules, especially since they could be prepared from any carbon-containing compound (Mandal and Das 2022). Moreover, the availability of an abundance of precursor molecules as well as approaches for the preparation of CDs (Mandal and Das 2022) has greatly complicated the comparison between different precursors and approaches in determining the most appropriate precursor and approach for scaling up and mass production for industrial applications. Hence, the dire need for standardization of the process could be understood. Furthermore, as CDs could even be prepared using municipal waste, there is a high possibility for the formation of toxic by-products, thereby requiring proper treatments for purification from the impurities. However, the failure of standardization of a proper purification protocol (Mandal and Das 2022) hinders further downstream processing and possibly leads to a dismissal of an alternative which could benefit in many ways, from the synthesis and multidisciplinary application of CDs as well as waste management. Apart from these, there is also a notable scarcity of studies on the antimicrobial activity of CDs against phytopathogens. When taking CNTs into

account, a considerable knowledge gap could be identified from the dearth of studies involving the antimicrobial properties of double walled CNTs, in addition to insufficient information on the functionalization of CNTs to reduce their cytotoxicity.

Despite being a cost-effective material with wide potential usage compared to carbon nanostructures, the reliability of these findings and long-term practical applications of NBC require further extensive studies and field trials to meet long-term needs and ensure its effectiveness (Liu et al. 2018). Utilizing NBC production can offer an economical and environmentally-friendly process compared to other carbon-based nanomaterials, due to its distinct physicochemical properties, abundant functional groups, and ease of surface modification, making it an emerging carbon-based material with versatile applications (Khare 2021). However, the information regarding the applications of NBC against microbial growth is comparatively limited when compared to the use of CDs and other carbon-based nanomaterials. This creates opportunities for researchers to conduct further investigations into the antimicrobial properties of these nanomaterials.

## 8 Conclusions

Carbon-based nanomaterials, such as CDs and GO, have demonstrated excellent antimicrobial properties, thereby recommending their application in a variety of fields such as for the control of phytopathogens and post-harvest disease control in agriculture, for nanotheranostics in medicine and extended shelf life in the food industry, as an eco-friendlier alternative to chemicals and antibiotics. However, there is also a wide scope for research to investigate their potential toxicity towards humans and the environment in the long term by understanding how a combination of different physicochemical characteristics influences the antimicrobial properties and nanotoxicity of these nanoparticles.

Compared to carbon nanostructures, NBC demonstrates promising potential as a sustainable and environmentally-friendly substance for antimicrobial applications. Its strong adsorption capabilities make it effective in removing and immobilizing pathogens and pollutants from soil and water. Additionally, NBC exhibits antibacterial properties, which can help prevent the development and spread of harmful pathogens. By providing a cost-effective and long-lasting solution for water and soil disinfection, NBC has the ability to significantly enhance public health and environmental quality. However, further research is needed to fully understand the mechanisms underlying its antimicrobial activity and

assess any potential environmental and human health impacts associated with its use.

### Abbreviations

AMR	Antimicrobial resistant
NBC	Nanobiochar
CDs	Carbon dots
GO	Graphene oxide
CNTs	Carbon nanotubes
ROS	Reactive oxygen species
MDR	Multi-drug resistant
PFRs	Persistent free radicals
RSBNPs	Rice straw biochar nanoparticles
FNPs	Fly ash nanoparticles
MNQ	2-Methoxyl-1,4-naphthoquinone
MIC	Minimum inhibitory concentration

### Acknowledgements

Not applicable.

### Author contributions

KN: Formal analysis, Methodology, Writing—original draft. ABF: Writing—original draft. SP: Writing—original draft, Writing—review & editing. PKCB: Supervision, Conceptualization, Writing—review & editing. MV: Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review & editing.

### Funding

Financial assistance by RG/2021/AG/02 grant received from the National Science Foundation, Sri Lanka.

### Availability of data and materials

All data in the manuscript are previously published.

### Declarations

#### Competing interests

No competing interests among authors.

#### Author details

<sup>1</sup>Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka. <sup>2</sup>Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka. <sup>3</sup>Sustainability Cluster, School of Engineering, University of Petroleum and Energy Studies, Dehradun, Uttarakhand 248007, India. <sup>4</sup>The UWA Institute of Agriculture, University of Western Australia, Perth WA6009, Australia.

Received: 10 July 2023 Revised: 6 November 2023 Accepted: 12 November 2023

Published online: 02 January 2024

### References

- Aftab ZEH, Aslam W, Aftab A, Shah AN, Akhter A, Fakhar U et al (2022) Incorporation of engineered nanoparticles of biochar and fly ash against bacterial leaf spot of pepper. *Sci Rep* 12(1):8561
- Al-Jumaili A, Alancherry S, Bazaka K, Jacob MV (2017) Review on the antimicrobial properties of carbon nanostructures. *Materials* 10(9):1066
- Anand A, Unnikrishnan B, Wei SC et al (2019) Graphene oxide and carbon dots as broad-spectrum antimicrobial agents—a minireview. *Nanoscale Horizons* 4(1):117–137
- Arfat YA, Ahmed J, Ejaz M, Mullah M (2018) Polylactide/graphene oxide nanosheets/clove essential oil composite films for potential food packaging applications. *Int J Biol Macromol* 107:194–203

- Arkan E, Barati A, Rahmanpanah M, Hosseinzadeh L, Moradi S, Hajjalyani M (2018) Green synthesis of carbon dots derived from walnut oil and an investigation of their cytotoxic and apoptogenic activities toward cancer cells. *Adv Pharm Bull* 8(1):149
- Aunkor MTH, Raihan T, Prodhan SH, Metselaar HSC, Malik SUF, Azad AK (2020) Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients. *Royal Soc Open Sci* 7(7):200640
- Azizi-Lalabadi M, Hashemi H, Feng J, Jafari SM (2020) Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites. *Adv Colloid Interface Sci* 284:102250
- Baruah J, Sahu D (2022) Comparative studies on carbon dots applications in plant systems. In *Carbon Dots in Agricultural Systems* (pp. 199–224)
- Castro HP, Souza VS, Scholten JD et al (2016) Synthesis and characterisation of fluorescent carbon nanodots produced in ionic liquids by laser ablation. *Chem A Eur J* 22(1):138–143
- Chausali N, Saxena J, Prasad R (2021) Nanobiochar and biochar based nanocomposites: advances and applications. *J Agric Food Res* 5:100191
- Chen J, Liu X, Zheng J et al (2013) Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Appl Soil Ecol* 71:33–44
- Chen X, Li W, Chen J (2022) Transcriptomics integrated with metabolomics reveals 2-methoxy-1, 4-naphthoquinone-based carbon dots induced molecular shifts in *penicillium italicum*. *J Fungi* 8(5):420
- Dadgostar P (2019) Antimicrobial resistance: implications and costs. *Infection and drug resistance*, pp.3903–3910
- Das R, Bandyopadhyay R, Pramanik P (2018) Carbon quantum dots from natural resource: a review. *Mater Today Chem* 8:96–109
- Dehvari K, Liu KY, Tseng PJ, Gedda G, Girma WM, Chang JY (2019) Sonochemical-assisted green synthesis of nitrogen-doped carbon dots from crab shell as targeted nanoprobe for cell imaging. *J Taiwan Inst Chem Eng* 95:495–503
- Deng LZ, Mujumdar AS, Pan Z et al (2020) Emerging chemical and physical disinfection technologies of fruits and vegetables: a comprehensive review. *Crit Rev Food Sci Nutr* 60(15):2481–2508
- Dizaj SM, Mennati A, Jafari S, Khezri K, Adibkia K (2015) Antimicrobial activity of carbon-based nanoparticles. *Adv Pharm Bull* 5(1):19
- Dong C, Lung S, Chen C et al (2019) Assessment of the pulmonary toxic potential of nano-tobacco stem-pyrolyzed biochars. *Environ Sci Nano* 6:1527–1535
- Dong X, Liang W, Meziani MJ et al (2020) Carbon dots as potent antimicrobial agents. *Theranostics* 10(2):671
- Ensafi AA, Sefat SH, Kazemifard N, Rezaei B, Moradi F (2017) A novel one-step and green synthesis of highly fluorescent carbon dots from saffron for cell imaging and sensing of prilocaine. *Sens Actuators, B Chem* 253:451–460
- Eskalen H, Çeşme M, Kerli S, Özjan Ş (2021) Green synthesis of water-soluble fluorescent carbon dots from rosemary leaves: applications in food storage capacity, fingerprint detection, and antibacterial activity. *J Chem Res* 45(5–6):428–435
- Esmailzadeh H, Sangpour P, Shahraz F, Hejazi J, Khaksar R (2016) Effect of nanocomposite packaging containing ZnO on growth of *Bacillus subtilis* and *Enterobacter aerogenes*. *Mater Sci Eng, C* 58:1058–1063
- Fan J, Claudel M, Ronzani C, Arezki Y, Lebeau L, Pons F (2019) Physicochemical characteristics that affect carbon dot safety: lessons from a comprehensive study on a nanoparticle library. *Int J Pharm* 569:118521
- Fang G, Liu C, Gao J, Dionysiou DD, Zhou D (2015) Manipulation of persistent free radicals in biochar to activate persulfate for contaminant degradation. *Environ Sci Technol* 49(9):5645–5653
- Frei A, Verderosa AD, Elliott AG, Zuegg J, Blaskovich M (2023) Metals to combat antimicrobial resistance. *Nat Rev Chem* 7:3
- Giraud L, Tourrette A, Flahaut E (2021) Carbon nanomaterials-based polymer-matrix nanocomposites for antimicrobial applications: a review. *Carbon* 182:463–483
- Gupta RD, Gupta SK (2016) Strategies for increasing the production of oilseed on a sustainable basis. In *Breeding oilseed crops for sustainable production* (pp. 1–18). Academic Press
- Gupta DK, Rajaura RS, Sharma K (2015) Synthesis and characterization of graphene oxide nanoparticles and their antibacterial activity. *Int J Environ Sci Technol* 1(1):16–24
- Gupta N, Debnath S, Sharma S, Sharma P, Purohit J (2017) Role of nutrients in controlling the plant diseases in sustainable agriculture. *Agriculturally Important Microbes for Sustainable Agriculture: Volume 2: Applications in Crop Production and Protection*, 217–262
- Gupta N, Gupta SM, Sharma SK (2019) Carbon nanotubes: synthesis, properties and engineering applications. *Carbon Letters* 29:419–447
- Gurtler JB, Mullen CA, Boateng AA, Mašek O, Camp MJ (2020) Biocidal activity of fast pyrolysis biochar against *Escherichia coli* O157: H7 in soil varies based on production temperature or age of biochar. *J Food Prot* 83(6):1020–1029
- Haider M, Cagliani R, Jagal J et al (2023) Peptide-functionalized graphene oxide quantum dots as colorectal cancer theranostics. *J Colloid Interface Sci* 630:698–713
- Jeyasubramanian K, Thangagiri B, Sakthivel A et al (2021) A complete review on biochar: production, property, multifaceted applications, interaction mechanism and computational approach. *Fuel* 292:120243
- Jiang M, He L, Niazi NK, Wang H, Gustave W, Vithanage M et al (2023) Nanobiochar for the remediation of contaminated soil and water: challenges and opportunities. *Biochar* 5(1):2
- Khare P (2021) A comprehensive evaluation of inherent properties and applications of nano-biochar prepared from different methods and feedstocks. *J Clean Prod* 320:128759
- Kościk I, Jankowski D, Jagusiak A (2021) Carbon nanomaterials for theranostic use. *C*, 8(1): 3
- Kousheh SA, Moradi M, Tajik H, Molaei R (2020) Preparation of antimicrobial/ultraviolet protective bacterial nanocellulose film with carbon dots synthesized from lactic acid bacteria. *Int J Biol Macromol* 155:216–225
- Kumar R, Kumar VB, Gedanken A (2020) Sonochemical synthesis of carbon dots, mechanism, effect of parameters, and catalytic, energy, biomedical and tissue engineering applications. *Ultrason Sonochem* 64:105009
- Li CL, Ou CM, Huang CC et al (2014) Carbon dots prepared from ginger exhibiting efficient inhibition of human hepatocellular carcinoma cells. *J Mater Chem B* 2(28):4564–4571
- Li JY, Liu Y, Shu QW et al (2017) One-pot hydrothermal synthesis of carbon dots with efficient up-and down-converted photoluminescence for the sensitive detection of morin in a dual-readout assay. *Langmuir* 33(4):1043–1050
- Li H, Huang J, Song Y et al (2018) Degradable carbon dots with broad-spectrum antibacterial activity. *ACS Appl Mater Interfaces* 10(32):26936–26946
- Li P, Sun L, Xue S et al (2022) Recent advances of carbon dots as new antimicrobial agents. *SmartMat* 3(2):226–248
- Lian F, Yu W, Zhou Q et al (2020) Size matters: nano-biochar triggers decomposition and transformation inhibition of antibiotic resistance genes in aqueous environments. *Environ Sci Technol* 54(14):8821–8829
- Lin X, Xiong M, Zhang J et al (2021) Carbon dots based on natural resources: synthesis and applications in sensors. *Microchem J* 160:105604
- Lin F, Wang Z, Wu FG (2022a) Carbon dots for killing microorganisms: an update since 2019. *Pharmaceuticals* 15(10):1236
- Lin R, Cheng S, Tan M (2022b) Green synthesis of fluorescent carbon dots with antibacterial activity and their application in Atlantic mackerel (*Scomber scombrus*) storage. *Food Funct* 13:4
- Liu W, Diao H, Chang H et al (2017) Green synthesis of carbon dots from rose-heart radish and application for Fe<sup>3+</sup> detection and cell imaging. *Sens Actuators, B Chem* 241:190–198
- Liu G, Zheng H, Jiang Z et al (2018) Formation and physicochemical characteristics of nano biochar: insight into chemical and colloidal stability. *Environ Sci Technol* 52(18):10369–10379
- Lou Y, Hao X, Liao L et al (2021) Recent advances of biomass carbon dots on syntheses, characterization, luminescence mechanism, and sensing applications. *Nano Select* 2(6):1117–1145
- Majidi HJ, Babaei A, Bafarani ZA et al (2019) Investigating the best strategy to diminish the toxicity and enhance the antibacterial activity of graphene oxide by chitosan addition. *Carbohydr Polym* 225:115220
- Mandal S, Das P (2022) Are carbon dots worth the tremendous attention it is getting: challenges and opportunities. *Appl Mater Today* 26:101331
- Miah G, Rafii MY, Ismail MR, Sahebi M, Hashemi FSG, Yusuff O, Usman MG (2017) Blast disease intimidation towards rice cultivation: a review of pathogen and strategies to control. *JAPS J Animal Plant Sci* 27(4)

- Mishra S, Singh A, Keswani C, Saxena A, Sarma BK, Singh HB (2015) Harnessing plant-microbe interactions for enhanced protection against phytopathogens. *Plant microbes symbiosis: applied facets*, 111–125
- Mittal J, Osheen S, Gupta A, Kumar R (2019) Carbon nanomaterials in agriculture. *Nanosci Sustain Agric* 153–170
- Monte-Filho SS, Andrade SIE, Lima MB, Araujo MCU (2019) Synthesis of highly fluorescent carbon dots from lemon and onion juices for determination of riboflavin in multivitamin/mineral supplements. *J Pharm Anal* 9:3
- Murmu SB, Mishra HN (2018) Post-harvest shelf-life of banana and guava: Mechanisms of common degradation problems and emerging counteracting strategies. *Innovative Food Science Emerging Technologies* 49
- Naghdhi M, Taheran M, Brar SK, Rouissi T, Verma M, Surampalli RY, Valero JR (2017) A green method for production of nanobiochar by ball milling-optimization and characterization. *J Clean Prod* 164:1394–1405
- Nair A, Mallya R, Suvarna V, Khan TA, Momin M, Omri A (2022) Nanoparticles—attractive carriers of antimicrobial essential oils. *Antibiotics* 11(1):108
- Newman MY, Abdullah J, Yusof NA, Abdul RS, Shueb RH (2021) Facile hydrothermal and solvothermal synthesis and characterization of nitrogen-doped carbon dots from palm kernel shell precursor. *Appl Sci* 11(4):1630
- Oleszczuk P, Ćwikła-Bundyra W, Bogusz A, Skwarek E, Ok YS (2016) Characterization of nanoparticles of biochars from different biomass. *J Anal Appl Pyrol* 121:165–172
- Omran BA, Baek KH (2022) Control of phytopathogens using sustainable biogenic nanomaterials: Recent perspectives, ecological safety, and challenging gaps. *J Cleaner Prod* 133729
- Pandey A, Devkota A, Yadegari Z, Dumenyo K, Taheri A (2021) Antibacterial properties of citric acid/ $\beta$ -alanine carbon dots against gram-negative bacteria. *Nanomaterials* 11(8):2012
- Pandit MA, Kumar J, Gulati S et al (2022) Major biological control strategies for plant pathogens. *Pathogens* 11(2):273
- Pramudita R, Gea S, Daulay A et al (2022) Synthesis of fluorescent citric acid carbon dots composites derived from empty fruit bunches of palm oil tree and its anti-bacterial property. *Case Stud Chem Environ Eng* 6:100277
- Pratiwi DC, Konhauser KO, Alessi DS (2022) Chapter 12—Biochar nanoparticles: interactions with and impacts on soil and water microorganisms, in *Biochar in Agriculture for Achieving Sustainable Development Goals*. Tsang DCWYS Ok, editors. Academic Press; p. 139–154.
- Rajapaksha AU, Chen SS, Tsang DC, Zhang M, Vithanage M et al (2016) Engineered/designer biochar for contaminant removal/immobilization from soil and water: potential and implication of biochar modification. *Chemosphere* 148:276–291
- Rajwade JM, Chikte RG, Paknikar KM (2020) Nanomaterials: new weapons in a crusade against phytopathogens. *Appl Microbiol Biotechnol* 104:1437–1461
- Ramanayaka S, Vithanage M, Alessi DS et al (2020a) Nanobiochar: production, properties, and multifunctional applications. *Environ Sci Nano* 7:11
- Ramanayaka S, Tsang DC, Hou D, Ok YS, Vithanage M (2020b) Green synthesis of graphitic nanobiochar for the removal of emerging contaminants in aqueous media. *Sci Total Environ* 706:135725
- Rashid MI, Shah GA, Iqbal Z et al (2023) Nanobiochar associated ammonia emission mitigation and toxicity to soil microbial biomass and corn nutrient uptake from farmyard manure. *Plants* 12(9):1740
- Raymaekers K, Ponet L, Holtappels D, Berckmans B, Cammue BP (2020) Screening for novel biocontrol agents applicable in plant disease management—a review. *Biol Control* 144:104240
- Razzaghi F, Obour PB, Arthur E (2020) Does biochar improve soil water retention? A systematic review and meta-analysis. *Geoderma* 361:114055
- Rimal V, Srivastava PK (2022) Synthesis and characterization of oil carbon dots. *Mater Today Proc* 65:2905–2908
- Romero MP, Alves F, Stringasci MD, Buzzá HH, Ciol H, Inada NM, Bagnato VS (2021) One-pot microwave-assisted synthesis of carbon dots and in vivo and in vitro antimicrobial photodynamic applications. *Front Microbiol* 12:662149
- Ruffo RS, Youssef K, Hashim AF, Ippolito A (2019) Nanomaterials as alternative control means against postharvest diseases in fruit crops. *Nanomaterials* 9(12):1752
- Salimi F, Moradi M, Tajik H, Molaei R (2021) Optimization and characterization of eco-friendly antimicrobial nanocellulose sheet prepared using carbon dots of white mulberry (*Morus alba* L.). *J Sci Food Agric* 101(8):3439–3447
- Saravanan A, Maruthapandi M, Das P, Luong JH, Gedanken A (2021) Green synthesis of multifunctional carbon dots with antibacterial activities. *Nanomaterials* 11(2):369
- Sattariazar S, Arsalani N, Ebrahimi SN (2023) Biological assessment of synthesized carbon dots from polyphenol enriched extract of pomegranate peel, incorporated with *Mentha piperita* essential oil. *Mater Chem Phys* 294:126981
- Shafiq F, Anwar S, Firdaus EB, Zhang L, Ashraf M (2023) Nano-biochar: Properties and prospects for sustainable agriculture. *Land Degradation and Development*
- Shahrzaman M, Hossain S, Ahmed T et al (2022) Chapter 7—Biological macromolecules as antimicrobial agents, in *Biological Macromolecules*. Nayak AK, AK DharaD Pal, editors. Academic Press p. 165–202
- Sidhu JS, Mayank PT, Kaur N, Singh N (2017) The photochemical degradation of bacterial cell wall using penicillin-based carbon dots: weapons against multi-drug resistant (mdr) strains. *ChemistrySelect* 2(29):9277–9283
- Toh SY, Loh KS, Kamarudin SK, Daud WRW (2014) Graphene production via electrochemical reduction of graphene oxide: synthesis and characterisation. *Chem Eng J* 251:422–434
- Vilaplana R, Chicaiza G, Vaca C, Valencia-Chamorro S (2020) Combination of hot water treatment and chitosan coating to control anthracnose in papaya (*Carica papaya* L.) during the postharvest period. *Crop Prot* 128:105007
- Vurro M, Miguel-Rojas C, Pérez-de-Luque A (2019) Safe nanotechnologies for increasing the effectiveness of environmentally friendly natural agrochemicals. *Pest Manag Sci* 75(9):2403–2412
- Wang Y, Zhu Y, Yu S, Jiang C (2017) Fluorescent carbon dots: rational synthesis, tunable optical properties and analytical applications. *RSC Adv* 7(65):40973–40989
- Wang MH, Li Q, Li X et al (2018) Effect of oxygen-containing functional groups in epoxy/reduced graphene oxide composite coatings on corrosion protection and antimicrobial properties. *Appl Surf Sci* 448:351–361
- Yaashikaa PR, Kumar PS, Varjani S, Saravanan A (2020) A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy. *Biotechnol Rep* 28:e00570
- Yang B, Zhang M, Wu M, Zhang H, Song Q, Yu S (2019a) Synthesis of biochar-based Cu<sub>2</sub>O nanoparticles and their antibacterial activity against *Escherichia coli*. *Inorganic and Nano-Metal Chemistry* 49(1):12–16
- Yang J, Gao G, Zhang X, Ma YH, Chen X, Wu FG (2019b) One-step synthesis of carbon dots with bacterial contact-enhanced fluorescence emission: fast Gram-type identification and selective Gram-positive bacterial inactivation. *Carbon* 146:827–839
- Zhang X, Wells M, Niazi NK et al (2022) Nanobiochar-rhizosphere interactions: implications for the remediation of heavy-metal contaminated soils. *Environ Pollut* 299:118810
- Zhao J, Cao X, Wang Z, Dai Y, Xing B (2017) Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae. *Water Res* 111:18–27
- Zhao C, Wang X, Yu L et al (2022) Quaternized carbon quantum dots with broad-spectrum antibacterial activity for the treatment of wounds infected with mixed bacteria. *Acta Biomater* 138:528–544
- Zhou Y, Qin S, Verma S, Sar T (2021) Production and beneficial impact of biochar for environmental application: a comprehensive review. *Biores Technol* 337:125451