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Abstract

The application of nanotechnologies to the agrifood sector provides one of the innovative tools able to support change in a system that, through its intensification and industrialization, currently fails both humans and the environment. The development of green nanotechnology, which exploits biological routes for the synthesis of nanomaterials, and specifically aims to minimize the production of hazardous substances and the input of energy, further underlines the disruptive potential of this technology. Due to the lack of toxic chemicals during their synthesis and to their high adaptability, green nanomaterials thus produced have a wide application domain, and this is reflected in the number of sustainable development goals affected by their use. This review describes the last trends on the eco-designed nanomaterials produced by means of green strategies for application in agriculture, such as nanofertilisers, nanopesticides, and nano(bio)sensors.

Keywords	green nanomaterials; nano(bio)sensors; nanofertilisers; nanopesticides; nanostructured biosensors; sustainable farming

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Dear Prof. Barcelò,

I am pleased to submit the review entitled *"Green nanoparticles and nanosystems fostering agrifood sustainability",* by Cecilia Bartolucci, Amina Antonacci, Fabiana Arduini, Danila Moscone, Leonardo Fraceto, Estefania Campos, Raouia Attaallah, Aziz Amine, Chiara Zanardi, Laura Cubillana, Jose Maria Palacios Santander, and myself for publication on *Trends in Analytical Chemistry* Journal, within the Virtual Special Issue "Biosensors for Agricultural and Food Safety".

The manuscript deals with the last trends on the design of green nanosystems to foster a more sustainable agriculture, where nanosystems entails both nanofertilisers/nanopesticides and nanosensors to be applied in smart farming. Hot topics are also considered regarding the 12 Principles of Green Chemistry as well as the Sustainable Development Goals, with the aim to boost people awareness on environmental protection.

I hope that this study will fit with the scope of the journal and can be considered for the publication on *Trends in Analytical Chemistry* Journal. The contribution is original and unpublished.

Thank you for your time and consideration.

Sincerely,

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Dear Editors and Reviewers,

We would like to thank you very much for your comments and suggestions for improving the manuscript. We tried to answer to all the comments and revised the manuscript taking into deep consideration all the suggestions. All the corrections were highlighted in red.

Reviewer 1

The review offers a good overview of the current state of the art regarding the application of green nanotechnologies to the agricultural field. The application of nanotechnologies to the agrifood sector provides one of the innovative tools able to support change in the industrial system.

The work is well organized, and it presents various types of nanoparticles and nanosystems, such as nanofertilisers, nanopesticides, and nanosensors, produced by means of green strategies, for application in agriculture. Numerous papers are reported, citing several types of nanoparticles synthetized using bioprocesses or, at least, employing renewable materials as starting blocks.

This review is a very good contribution to the field and should be published in TRAC after minor revisions.

My only objection is that all the experiments reported were performed *in-vitro* or in green-house conditions. It is thus difficult to evaluate the effectiveness of the proposed nanomaterial-based remediation techniques when applied to real agriculture situations (effect of weather, different soil composition), especially regarding the long-term techniques proposed, such as parasites removal, fertilization and growth-induction of crops. It is my opinion, if possible, some research articles regarding these points should be added to the review.

Thank you for the comment. Indeed, it is absolutely true that the evaluation of the effectiveness of such green nanoformulations in field conditions has not yet been carried out, according to the literature. Thus, further studies are required to better highlight this concern. For this reason, we added the following sentence: "Despite the numerous advantages and potentials of nano-based formulations for agriculture applications, recent concerns have been highlighted about the lack of studies under field conditions. As pointed out by Lowry (2019), there is a crucial requirement to better understand the mechanism promoting adverse response of the use of nanoformulations under biotic (plant pathogens, insects, and weeds) and abiotic (temperature, drought, flooding, and salinity) stressors. Indeed, a broad study in field conditions become essential to generate comprehensive data, in support of future decision making and appropriate regulatory tools".

Some more specific comments are:

The introduction is repetitive, and some sentences could be cancelled, however it would be important to add more specific needs of the agriculture sector.

Thank you for the comment. The introduction was revised accordingly, by deleting some sentences, as well as adding some others to better highlight the agricultural sector requirements, as suggested.

Page 4 line 13-14: phrase not clear (The prevailing present agricultural practices intensify factors that negatively influence it).

Thank you for the comment, the sentence was changed accordingly.

Page 5, line 4: phrase not clear (enhancing crop productivity while reducing the needed amount)

I strongly recommend this review for publishing in TRAC.

Thank you for the comment, the sentence was changed accordingly.

Reviewer 2

The review covers recent advances in a very emerging and interesting topic, related to the green chemistry focused on nanoparticles synthesis and its application in the agrifood field as well as for biosensing approaches.
The authors have performed an exhaustive bibliographic revision and rightly summarized it in a reader-friendly mode. The tables summarizing the most representative works are also highly appreciated by the reader. I also appreciate the authoritative opinion/discussion given in the section "Gaps, obstacles, and trends of green nanoparticles and nanosystems".

However, some important points should be improved before its publication at TRAC journal:

1. The major point in my opinion is the poor analytical focus of the review. Only the short section devoted to biosensing applications is of high interest for the readers of TRAC journal. I would suggest to re-organize the review (starting by the title), giving priority to the analytical applications of the nanoparticles prepared by green chemistry. In this sense, i.e. optical biosensors are missing in the article (authors only focus on electrochemical ones).
Thank you for the precious comment. We changed the title in "Green nanomaterials fostering agrifood sustainability", because the old title was able confusing the reader about the nanosystems, here entailed as nanofertilisers, nanopesticides, and nano(bio)sensors. The abstract was also edited accordingly. The section describing the nano(bio)sensors was enriched to better point on the analytical aspect of the review, as well as the optical nanosensors added (all highlighted in red). Regarding the re-organisation of the structure of the review, we think that the explanation of the nanomaterials obtained by the green synthesis acquires a greater meaning if reported before the section on nanobiosensors, which are precisely designed using these green nanomaterials.

2. More figures/illustrations of the different analytical/biosensing approaches would also be highly appreciated.
Thank you for the comment. As suggested, a figure was added, reporting the illustration of the most representative biosensors described in the section.

3. At the caption of figure 1, the meaning/explanation of the number of the different blocks is missing.
Thank you for the comment. The meaning of the blocks refers to the official final list proposed by the UN "Final list of proposed Sustainable Development Goal indicators" in the report of the Inter-Agency and Expert Group on Sustainable Development Goal Indicators. We added the specific reference.

4. At table 3, analytes and biosensing principles/approaches are missing.
Thank you for the comment. The table was enriched by adding, as suggested, the biosensing configuration, the transduction mechanism, and the target analyte.

Highlights

- Nanotechnology has great potential impacts on food and agriculture, with the ambition to reduce amount of spread chemicals, through better targeted and controlled use of inputs.
- Green nanomaterials contribute to the development of eco-designed nanoparticles and nanosystems supporting more sustainable applications in the agrifood sector.
- Despite the availability of green synthetic strategies, existing knowledge gaps on potential toxicity of nanomaterials, require concerted efforts on assessing risk and evaluating effects on human health and on the environment.

Green nanomaterials fostering agrifood sustainability

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Abstract
The application of nanotechnologies to the agrifood sector provides one of the innovative tools able to support change in a system that, through its intensification and industrialization, currently fails both humans and the environment. The development of green nanotechnology, which exploits biological routes for the synthesis of nanomaterials, and specifically aims to minimize the production of hazardous substances and the input of energy, further underlines the disruptive potential of this technology. Due to the lack of toxic chemicals during their synthesis and to their high adaptability, green nanomaterials thus produced have a wide application domain, and this is reflected in the number of sustainable development goals affected by their use. This review describes the last trends on the eco-designed nanomaterials produced by means of green strategies for application in agriculture, such as nanofertilisers, nanopesticides, and nano(bio)sensors.

Keywords: green chemistry, nano(bio)sensors, nanofertilisers, nanopesticides, sustainable farming

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1. Introduction

1.1 Sustainable food and agriculture

Current food systems are not sustainable and the need for a new approach to food production, distribution, and consumption is a key goal, eventually underlined by many. In particular, there is a need to adopt new agricultural practices, which provide less soil degradation, sustainable use of resources, maintenance of biodiversity, and support of agricultural ecosystem and ecosystem services, while ensuring food security and a healthy nutrition for all. Presently used agricultural approaches often do not allow to pursue these goals simultaneously (e.g. agricultural intensification and maintenance of biodiversity), triggering a spiralling negative effect. Furthermore, agrochemicals necessary to obtain higher yields are responsible for increased pathogens and pest resistance, reduced biodiversity, diminished nitrogen fixation, and pollination decline. In addition, pesticides are the most energy-intensive agricultural inputs, and nitrogen fertilizers form the largest of all indirect energy inputs to agriculture, while agriculture itself is responsible for 10-12% of total greenhouse gas emissions [1]. There is a strong interlinkage between agriculture and climate change. In a detrimental vicious cycle, the prevailing present agricultural practices intensify factors that negatively influence climate change, while growing environmental pressure and changing biophysical conditions affect both quality and quantity of food production, causing increased food insecurity, and hampering adequate nutrition and equitable food distribution [2].

Growing attention to the need of a change in the system is reflected in the 2030 Agenda for Sustainable Development and specifically in the Sustainable Development Goal (SDG) 2, “End hunger, achieve food security and improved nutrition and promote sustainable agriculture”. Efforts to meet SDG 2 will also contribute to reach other SDGs underlining the integrated approach of the 2030 Agenda. A transformation, however, relies on more than 140 countries prioritizing, aligning and integrating SDGs into national policies, plans, budgets, and cooperation efforts [3].

1.2 Nanotechnology and its potential impacts on agriculture

Progress toward a more sustainable agriculture requires the acquisition of new knowledge and the development of new technologies supporting, among others, more environmentally-friendly formulations. In this regard, nano-based approaches have the potential to provide effective solutions to multiple problems faced by global agriculture, as indicated by an increasing number of studies reporting the application of nanotechnology in the agricultural sector [4–6].

In particular, site-targeted controlled delivery of active substances, achieved through nano-based systems, improves efficiency, enhancing crop productivity with a reduced amount of inputs [4]. The development of nanosensors also contributes to the improvement of soil quality and plant health [7]. Similar (bio)sensors entails the use of nanomaterials, including nanotubes, nanowires, nanoparticles or nanocomposites, to boost their analytical performances in terms of sensitivity and stability, among others. Indeed, nanomaterials possess unique thermal, electrical and optical features, able to provide benefits to the overall efficiency of these biosensing systems.

However, a constant difficulty concerns the need to produce these formulations without employing toxic compounds during synthesis [8,9]. Until recently, nano-based formulations for agricultural applications were synthesized by chemical, physical, and biological techniques, which tend to be expensive and can lead to environmental toxicity, cytotoxicity, and carcinogenicity [8,9]. Toxicity results mainly from substances such as organic solvents, reducing agents, and stabilizers used to avoid undesirable colloid agglomeration. There is therefore a need to develop and improve methods for the efficient, non-hazardous, and economic production of nano-based compounds and materials, in order to encourage their use in agriculture.

1.3 Green nanomaterials in connection with the SDGs

In almost 20 years of life, green chemistry showed numerous advances and contributions in the agrochemical sector. A critical review, highlighting the Green Chemistry evolution and showing progress in academic research as well as in processes implementation at industrial or entrepreneurial levels, was recently published by Anastas et al. [10]. In this paper, the authors explore activities within the 12 Principles of green chemistry and discuss its benefits, emphasizing the progress made towards meeting the 2030 Agenda.

Of particular interest is nanobiotechnology, which exploits biological routes for the synthesis of nanoparticles, employing bacteria, fungi, algae, as well as plant extracts or specific biomolecules from plants (including carbohydrates, proteins, and lipids) to synthesize nanoparticles, using nontoxic and inexpensive materials, while ensuring minimal byproduct formation and low energy consumption [11]. The increasing interest for green nanotechnology underlines the potential provided by the synthesis and application of green nanomaterials [12,13] in many different fields, such as agriculture and biomedicine [14], environment [15], food [16], energy [17], and building [18], in line with SDG 12 “Ensure sustainable consumption and production patterns” (Figure 1) [9].

Recently, sonochemistry and microwave irradiation consolidated their status as green, clean, and environmentally friendly methods of synthesis of green (nano)materials and have been included in the Green ChemisTREE of Anastas et al. [10], connected to the branch which represents the green chemistry principle ‘Design for energy efficiency’. Energy efficiency is not the only advantage provided by both techniques: atom economy (i.e. capacity to be adjusted or applied to lower volumes/amount and to provide high yields), drastic time saving, low cost, simple instrumentation compared with other technologies, waste prevention, use of benign solvents, etc., should be highlighted as well. In the past few years, many examples of the use of ultrasound and microwave for synthesizing green (nano)materials can be found in the literature: *i.e.*, nanorods, nanosheets, and nanotubes (1D and 2D nanomaterials) [19] using microwave; and nanoparticles (OD nanomaterials) [20] and graphene (2D nanomaterials) [21], among many others, using ultrasound-assisted synthesis. Nanocomposites can also be obtained in an ecological way [22] and, recently, a study regarding the synthesis of green nanomaterials (quantum dots), combining sonochemistry and microwave irradiation, has been reported [23].

While the production of green nanomaterials generally supports the accomplishment of SDG 12, the use of these materials in the agrifood sector can be directly connected with SDG 2. However, strong interlinkages between all SDGs show how the application of green nanomaterials to other sectors, such as medicine (SDG 3), water treatment (SDG 6), energy (SDG 7), and construction building (SD 11), can create synergies, which indirectly contribute to addressing SDG 2. The production of innovative, environmentally friendly nanomaterials also contributes to meeting SDG 13 and 14, and indirectly supports SDG 8 and 9 through the economic contribution provided by the development of industries and infrastructures, aimed at a sustainable growth (Figure 1). It is important, however, that current technological advances are implemented through public and private investments, supporting an innovation that can provide real benefits.

Figure 1. Overview of the connections between SDGs and green nanomaterials for a sustainable world. The numbers within the boxes refer to the final list of SDGs indicators proposed by the UN [24].

2. Green nanoparticles and nanosystems

2.1 Green chemistry to design next-generation nanoparticles for crop protection and plant development

2.1.1 Production of nanoparticles from microorganisms and plants

The production of nanoparticles using plants and microorganisms has attracted increasing attention from scientists worldwide, due to the eco-friendly nature and simplicity of these production processes, compared to other routes [25,26]. Many bacteria and plants have been used to produce different types of nanoparticles for the purpose of crop protection. For example, extracellular synthesis of Ag/AgCl nanoparticles was performed using exudate from the fungus *Macrophomina phaseolina.* The nanoparticles showed strong inhibitory effects on the growth kinetics of both gram-positive and gram-negative bacteria strains. In addition, these nanoparticles could be used for seed protection, since no effects on seed germination were observed after treatment *in vitro* using filter paper in lab conditions (Whatman filter paper size 41) [27]. In another work, nanoparticles were synthesized using a cell wall polymer from the fungus *Fusarium oxysporum* f.sp. *lycopersici*. The fertilizer activity of the nanoparticles was evaluated using foliar applications to tomato plants challenged with *F. oxysporum* f.sp. *lycopersici*, under greenhouse conditions. The treated plants showed delayed development of disease symptoms, as well as reduced disease severity. In addition, the treatment with the nanoparticles increased the number of flowers and the yield of fruit [28]. In another example, extracellular synthesis of chitosan nanoparticles was performed using proteins extracted from the endophytic fungus *Penicillium oxalicum*. Chickpea seeds treated with the nanoparticles showed a higher seed germination rate, higher seedling vigor index and biomass, and increased shoot and root elongation, compared to untreated control plants. The nanoparticles also inhibited the growth of phytopathogens such as *Pyricularia grisea*, *Alternaria solani, and Fusarium oxysporum* under *in vitro* lab conditions [29].

Cell-free extracts of *Trichoderma viride* were employed to synthesize silver nanoparticles, which were tested *in vitro* and *in vivo* for the control of *Alternaria solani*, the causative agent of early blight disease in tomato plants. The *in vitro* antifungal activity tests showed a 100% reduction of the spore count after 3 days of treatment, as well as 74% reduction of the fungal biomass after 7 days. Foliar application of the nanoparticles (under greenhouse conditions) led to a 48.57%

decrease of the fungal spore count, while the fresh weight and the total chlorophyll content of the plants increased by 32.58 and 23.52%, respectively, compared to infected untreated plants [30].

Biosynthesized silver nanoparticles produced using an indigenous isolate of *Trichoderma viride* showed good antifungal activity when tested *in vitro* against the tea pathogen fungus *Phomopsis theae* [30]. Biosynthesis of silver nanoparticles was also performed using *Stenotrophomonas* sp. isolated from agricultural soil, with evaluation of the biological activity of the nanoparticles against foliar phytopathogens (*Alternaria alternata*, *Curvularia lunata*, and *Bipolaris sorokiniana*) and a soil-borne phytopathogen (*Sclerotium rolfsii*). The *in vitro* application of the biosynthesized nanoparticles at concentrations of 2, 4, and 10 μg/mL led to 100% inhibition of conidial germination, for all the foliar phytopathogens tested, while total conidial germination was observed for the control.

In another work, the suppression of *S. Rolfsii* in chickpea plants was observed after 24 h of pathogen challenge, under greenhouse conditions [31]. The entomopathogenic fungus *Beauveria bassiana* was used to synthesize silver nanoparticles, whose bioefficacy was evaluated *in vitro* against the mustard aphid (*Lipaphis erysimi* Kalt). Among the 25 *B. bassiana* isolates tested, two isolates caused the highest mortality rates of 90 and 64% against mustard aphid [32]. The biological activity of zinc oxide nanoparticles coated with *Bacillus thuringiensis* was evaluated against the pulse beetle *Callosobruchus maculatus*, with dose-dependent activity observed in terms of fecundity and hatchability. A mortality rate of 100% was obtained using the nanoparticles at a concentration of 25 μg/mL under *in vitro* conditions, while the LC_{50} was estimated to be 10.71 μg/mL. The nanoparticles also decreased enzymatic activity (mid-gut α-amylase, cysteine protease, α-glucosidase, and glutathione S-transferase) in *C. maculatus* [33].

Plant exudates, such as latex, from different plants have been used to synthesize silver nanoparticles [34–37]. The nematicidal activity of silver nanoparticles synthesized using latex from *Euphorbia tirucalli* was evaluated against the root-knot nematode (*Meloidogyne incognita*). *In vitro* assays showed that the silver nanoparticles were lethal to the second stage juveniles of *M. incognita* and inhibited egg hatching. *In vivo* assays using tomato plants under greenhouse conditions showed a significant reduction in root infestation when the plants were treated with the silver nanoparticles, together with healthier plant growth [38]. The saponin fraction of *Eclipta alba* was used to synthesize zinc oxide nanoparticles, which were evaluated as a growth promoter and for the management of downy mildew in pearl millet. The treated plants showed a 35% reduction of disease incidence, compared to the untreated control (green house conditions). In addition, treatment with the nanoparticles significantly increased defense enzymes, seed germination, and seedling vigor under lab and greenhouse conditions [39]. In another example, zinc oxide nanoparticles were synthesized using an extract from the alga *Halimeda tuna*. Phytochemical-capped zinc oxide nanoparticles with P supplementation increased the growth and total biomass of *Gossypium hirsutum* (cotton) by 131%, compared to untreated control plants (greenhouse conditions). In addition, there were increases of the contents of photosynthetic pigments and total soluble proteins [40]. These biological routes have employed different types of plant extracts to produce a variety of nanomaterials. Table 1 summarizes the most promising studies of nanoparticles prepared using plant-based biological routes, in the agricultural field.

Table 1. Summary of different types of nano-based formulations synthesized using biological routes employing plants.

Nanomaterial	Properties	Plant	Main results	Reference
Zinc oxide nanoparticles	MD: 28 nm; spherical shape	*Parthenium hysterophorus*	Positive effects on growth and yield parameters were observed in *Arachis hypogaea* plants treated with up to 300 ppm of zinc oxide nanoparticles in zinc-deficient soil under greenhouse conditions.	[41]
Zinc-chitosan nanoparticles	–	–	After treatment of two varieties of wheat plants (*Triticum durum*) twice a week during five weeks under lab conditions, the concentrations of zinc in the grain increased by about 27 and 42% for cultivars MACS 3125 and UC1114, respectively, compared to the control (lab conditions).	[42]
Zinc oxide nanoparticles	MD: 23.3 nm	*Musa paradisiaca*	Treatment with zinc oxide nanoparticles at 50 µg/mL in lab conditions caused 42% mortality after 24 h, while zinc acetate caused 80% mortality. The treatment led to the accumulation of nanoparticles in the gut of *Ceriodaphinia cornuta*, together with structural deformities (lab conditions).	[43]
Silver nanoparticles	MD: 67.94-133.2 nm; PDI: 0.156-0.272; ZP: -19.6 - -22.8 mV	*Azadirachta indica*	The antibacterial activity (*in vitro)* of synthesized silver nanoparticles was evaluated against the plant pathogen *Xanthomonas oryzae* pv. *Oryzae*. The best antibacterial activity of the nanoparticles was observed using 5 mL of	[44]

			plant extract and 10 min exposure to sunlight (lab conditions).	
Silver nanoparticles	MD: 25-40 nm; ZP: -23.8 mV	*Phyllanthus emblica*	*In vitro* administration of 10 mg/L of phytochemical-capped silver nanoparticles increased the number of seedlings of wheat, due to protection against oxidative stress, with increased biomass, greater root elongation, and higher root cell viability.	[45]
Chitosan-silver nanoparticles	MD: 17.26 (TEM) and 117.9 (DLS); spherical shape	*Alpinia galanga* L.	The nanoparticles were tested as an antifungal formulation against *Pyricularia oryzae in vitro*. Association with Trihexad 700 WP potentiated the fungicidal activity of the chitosan-silver nanoparticles. The inhibition zones were 25 and 12 nm for nanoformulations with and without Trihexad, respectively.	[46]
Acacia gum-silver nanoparticles	MD: 16.7 nm (TEM) and 22 nm (DLS); spherical shape	*Acacia senegal*	The effects of the nanoparticles on the growth and yield of two varieties (Bronco and Nebraska) of common bean (*Phaseolus vulgaris*) were evaluated after foliar application (5-60 ppm) using pot experiment. All concentrations tested increased root length, number of leaves, leaf area, plant height, total dry and fresh weights, and yield. In addition, both species showed alterations of protein patterns, while the Nebraska variety showed altered phytohormone balance.	[47]
Silver nanoparticles	MD: 2-74 nm; hexagonal shape	*Gracilaria foliifera*	100% multiple shoot bud regeneration and two-fold higher shoot elongation were observed in plants of *Alternanthera sessilis* L. supplemented with 2.5 mg/L of nanoparticles in combination with 50 mg/L adenine sulphate under *in vitro*.	[48]

				[49]
Gold nanoparticles	MD: 10-30 nm; spherical or nearly spherical shape	*Alpinia galanga* L.	Activation of germination was observed using 5 and 10 ppm of phytochemical-capped gold nanoparticles under greenhouse conditions. A 3-fold increase of seedling vigor was obtained, compared to the untreated control. The best physiological and biochemical properties of maize plants were achieved using 10 ppm of the nanoparticles.	
Gold nanoparticles	MD: 80-120 nm; ZP: -41.4 mV; spherical shape	*Alternanthera bettzickiana*	Slight inhibition of hatching was observed after exposure of embryos of *Danio rerio* to 25 μM of gold nanoparticles in lab conditions. Further increase of the concentration (to 50 μM) led to strong inhibition of hatching (lab conditions).	[50]
Copper oxide nanoparticles	MD: 40-50 nm (DLS) and 20-30 nm (TEM)	*Morus alba*	*In vitro* biological activity was evaluated considering the metabolism and antioxidant activity of *Solanum lycopersicum* and *Brassica oleracea* L. var. *botrytis*. The highest concentrations tested (100 and 500 mg/L) significantly decreased the total chlorophyll and sugar contents in both species, while at 10 mg/L, the tomato plants presented slightly increased pigment and sugar contents. Increases of lignin deposition and antioxidant enzyme activities showed concentration-dependent profiles.	[51]

Copper-chitosan nanoparticles	MD: 150 nm; ZP: +22.6 mV	–	Significantly increased growth parameters were observed in maize plants when seeds were treated with 0.04-0.12% copper-chitosan nanoparticles, compared to untreated controls under *in vitro*. At the same concentrations, the nanoparticles also increased enzyme activity and the total protein content in germinating seeds.	[52]
Copper-chitosan nanoparticles	MD: 361.3 nm; PDI: 0.20; ZP: +22.1 mV	–	Experiments using pots and under field conditions, with application of copper-chitosan nanoparticles at concentrations of 0.04-0.16% and 0.12-0.16%, respectively, using seed and foliar treatments, led to increases of growth parameters, antioxidant activity, enzyme defenses, and photosynthetic pigments in maize plants.	[53]
Sulfur nanoparticles	MD: 20 nm; spherical shape	*Melia azedarach*	The growth regulator effect of sulfur nanoparticles was evaluated in the growth and development of *Cucurbita pepo* under greenhouse conditions. All concentrations tested (100-400 ppm) increased the numbers of leaves and branches, stem diameter, and plant height, under field conditions. There were concentration-dependent increases of root and shoot elongation. Concentrations higher than 600 ppm had no effect on plant growth.	[54]
Sulfur nanoparticles	MD: 40 nm; spherical shape	*Ailanthus altissima*	The effect of the nanoparticles on growth of tomato plants was evaluated in greenhouse. Increased concentration-dependent growth (root and shoot lengths) was observed using the sulfur nanoparticles at 100-300 ppm. However, higher concentrations (400 and 600 ppm) resulted in inhibitory effects.	[55]

MD: mean diameter; PDI: polydispersity index; ZP: zeta potential.

To the above mentioned advantages provided by the green synthesis of nanoparticles, some compounds present in plants or their extracts can act both as reducing and capping agents, generating stable nanoparticles and avoiding one of the separate steps required in physicochemical processes [56,57]. However, although microorganisms have been extensively used to produce biogenic nanoparticles, there are some gaps that need to be addressed. The reduction mechanisms that microorganisms use to synthesize nanoparticles are not fully understood, making it hard to control the processes. In addition, it is difficult to maintain the stability of culture media, since conditions such as pH, salinity, and temperature of the medium can influence the reaction process, which hinders scaling up to an industrial level. Furthermore, the use of plants to synthesize biogenic nanoparticles also has some drawbacks, such as dramatic changes in size and shape of nanoparticles, depending on which part is used for synthesis, extraction, isolation and purification of nanoparticles; low recovery due to decreased synthetic rate of nanoparticles, dependent on plants' variable yields of secreted proteins [58,59]. It is necessary to improve knowledge concerning the selection of suitable plant extracts for use as reducing agents, as well as concerning the mechanisms of the reduction processes. In summary, it is necessary to acquire a deeper understanding of the mechanisms governing the synthesis of nanoparticles in live organisms, in order to assist in the development of cost-effective approaches for the industrial synthesis of nanoparticles with appropriate physicochemical properties, high biological activity, and low toxicity [60]. Figure 2 reports the different sources for the green synthesis of nanoparticles and the diverse applications in the environmental field. Despite the numerous advantages and potential shown by nano-based formulations for agriculture applications, recent concerns have been expressed about the lack of studies under field conditions. As pointed out by Lowry [61], there is a crucial need to better understand the mechanism promoting both favourable as well as adverse responses, enticed by the use of nanoformulations under biotic (plant pathogens, insects, and weeds) and abiotic (temperature, drought, flooding, and salinity) stressors. A broad study in field conditions becomes essential to generate comprehensive data, in support of future decision making and development of appropriate regulatory tools [4].

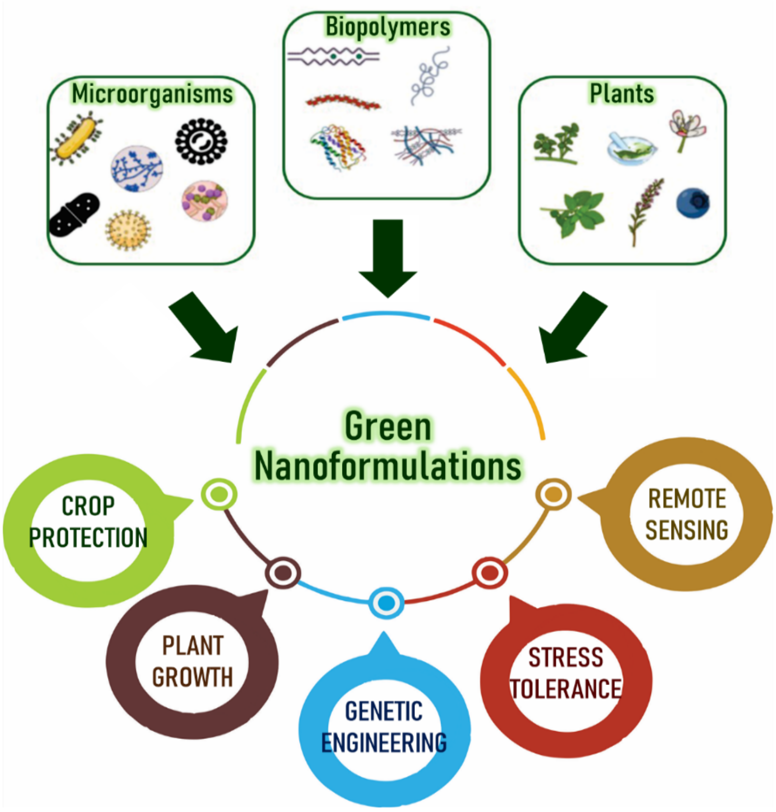


Figure 2. Green synthesis of nanoparticles using microorganisms and plants and applications in the environmental field.

2.1.2 Biopolymers as nanocarriers and nanoencapsulation devices

Biopolymers are versatile materials obtained from natural resources and are widely used for the development of nanocarriers. They offer desirable properties such as biodegradability, biocompatibility, good stability, semi-crystallinity, and non-toxicity [62,63]. The sources of biopolymers include microorganisms, algae, plants, and animals [62]. An important biopolymer is chitin, a natural polysaccharide found in the exoskeletons of insects, some hard structures of invertebrates and fish, and the cell walls of fungi. The deacetylation of chitin produces chitosan, a linear polycationic polymer composed of β-1,4-2-deoxy-2-amino-D-glucopyranose and β-1,4-2-deoxy-2-acetamido-D-glucopyranose glycosidic linkages [64].

Chitosan-based nanocarriers have a wide range of applications in sustainable agriculture, such as crop protection [65,66] and improved plant growth [67,68]. For example, the application of chitosan nanoparticles loaded with thiamine was evaluated relative to growth and protection of a chickpea crop. Treatment of the seeds increased germination, growth and indole acetic acid production of chickpea seedlings, compared to untreated seeds. In addition, the induction of defense enzymes in the leaves and roots of the plants was observed after foliar treatment with the synthesized nanoparticles [69]. The fungicidal activity of thiamine-chitosan nanoparticles against *Fusarium graminearum* in wheat plants was evaluated under *in vitro* and greenhouse conditions. The greatest decrease in growth of the fungus was 77.5%, achieved using 5000 ppm of chitosan nanocarriers. In greenhouse experiments, the areas under the disease progression curves were 3.34 and 7.7 for plants treated with thiamine-chitosan nanoparticles and untreated control plants, respectively, 28 days after inoculation [70].

The antifungal activity of oleoyl-chitosan nanoparticles was investigated against the pathogenic fungus *Verticillium dahliae*. Use of the synthesized nanoparticles at 2 mg/mL in the medium led to a significant decrease of mycelium growth. In addition, the morphologies of the spores and hyphae were altered following treatment with the nanoparticles [71]. In another work, foliar delivery of 10-100 mg/L chitosan nanoparticles loaded with NPK fertilizer was investigated using wheat plants. Fertilization of the wheat plants using chitosan-NPK decreased the plant lifecycle by 40 days, compared to normally fertilized plants. In addition, the grain yield increased by more than 50% in wheat plants treated with the chitosan-NPK nanoparticles, compared to untreated control plants [72].

Another useful biopolymer is lignin, the second most abundant component of terrestrial plants, composed by propyl phenol units (coniferyl alcohol and sinapyl alcohol, with a smaller amount of p-coumaryl alcohol) [73]. Lignin nanoparticles were synthesized using lignin extracted from two sugarcane bagasses. The near-spherical nanoparticles showed good ability to stabilize Pickering emulsions used to encapsulate curcumin. Organosolv lignin was found to provide greater stability, compared to alkaline lignin, with retention of 73% of the encapsulated curcumin after 96 h [74]. Calcium alginate nanoparticles loaded with cypermethrin were designed for the release of this broad-spectrum insecticide. The results indicated that this nanoformulation could be a safe system for the sustained and slow release of cypermethrin, decreasing the environmental damage caused by its excessive use [75]. The nanoencapsulation of biomolecules in protein-based nanoparticles has attracted much attention, due to the presence of different functional groups on the particle surfaces. These groups allow interaction with many different molecules, enabling the encapsulation of both hydrophobic and hydrophilic compounds [76]. Table 2 summarizes the most promising nano-based formulations based on biopolymers and proteins, for use in agricultural applications.

Table 2. Summary of different types of nano-based formulations produced using natural polymers.

Nanomaterial	Properties	Main results	Reference
Whey protein-pectin nanoparticles	MD: 160 nm; ZP: -0.53 mV; spherical shape	D-limonene was encapsulated in whey protein and pectin nanoparticles by a complexation method. The highest complex formation was obtained at a ratio of 4 to 1 between whey protein and pectin.	[77]
Sodium alginate nanoparticles	–	Sodium alginate nanoparticles with or without emulsifiers (Tween 20 or glycerol monostearate), loaded with clove essential oil, were synthesized and their biological activities were evaluated against *S. aureus* and *S. typhimurium*. Nanoencapsulation potentiated the antibacterial activity of clove essential oil against both bacteria *in vitro*.	[78]
Zein-caseinate-pectin nanoparticles	MD: 150 nm; PDI: 0.16; spherical shape	Eugenol was encapsulated in zein-caseinate-pectin nanoparticles using a nano spray-drying method. The synthesized nanoparticles presented a spherical shape and a uniform size distribution, making them good candidates for applications in the agricultural field.	[79]
Neem gum nanoformulations	MD: 20-40.83 nm; spherical and uneven nanoparticles	Neem gum nanoformulations prepared using *Azadirachta indica* extract were used as larvicide, pupicide, and anti-feedant against *Helicoverpa armigera* and *Spodoptera litura*. At 100 ppm, the larvicidal activities against fourth instar *H. armigera* and *S. litura* were 92.46 and 86.80%, respectively. At the same concentration, 100% pupicidal and anti-feedant activities were observed for both species studied. The results suggested that these nanoparticles could be used as novel biopesticides for crop protection.	[80]
Zein nanoparticles	MD: 142.5-205.2 nm; PDI: 0.330-0.442; ZP: -12.8 - -20.2	The repellent activities of zein nanoparticles loaded with geraniol and R-citronellal were evaluated *in vitro* against *Tetranychus urticae*. At 5 mg/mL, zein nanoparticles loaded with geraniol or R-citronellal showed 27.8 and 76.3% repellency, respectively. After dilution (10- and 100-fold), the repellency of the geraniol formulations remained at the same level, while the biological activity of the R-citronellal formulations decreased significantly.	[81]
Zein nanoparticles	MD: 282-302 nm; PDI: 0.34-0.52; ZP: -15 - 43 mV	Zein nanoparticles were used to co-encapsulate two combinations of active agents (geraniol + eugenol; geraniol + cinnamaldehyde). Biological activity was evaluated *in vitro* (using *Chrysodeixis includens*) and under field	[82]

		conditions (using *Tetranychus urticae*). Semi-field assays showed that the repellent activity of the nanoparticles increased over time, while the non-encapsulated compounds showed higher repellency activity against the mite in the first two hours. Similarly, the non-encapsulated compounds showed higher larvicidal activity against soybean looper moth, while greater sublethal effects (considering larval and pupal masses) were obtained with the encapsulated compounds.	
Zein nanoparticles	MD: 278 nm; PDI: 0.31; ZP: -36 mV; spherical shape	Neem oil was encapsulated in zein nanoparticles and the toxicological activity of the formulation was evaluated. Nanoencapsulation of the neem oil reduced its genotoxic effect about 8-fold, compared to the non-encapsulated oil. Encapsulation of the neem oil avoided effects on the pharyngeal pumping activity of the soil nematode *C. elegans*.	[83]
Zein nanoparticles	MD: 231.5 nm; ZP:-17.52 mV	Limonene was encapsulated in zein-sodium caseinate nanoparticles and its biological activity was evaluated against the plant pathogen bacterium *Pseudomonas syringae*. Nanoencapsulated limonene showed higher antimicrobial activity towards *P. syringae*, compared to the non-encapsulated compound.	[84]
Cyclodextrin and nanofibers	-	Dose-dependent inhibition of fungal growth was observed with treatment using a β-CD inclusion complex. A β-CD-cinnamon essential oil inclusion complex was more effective against fungi, compared to a β-CD-oregano essential oil inclusion complex, at all the concentrations tested. Superior activity was observed for chitosan/PVA/β-CD-EO nanofibrous films, compared to the inclusion complex.	[85]
Chitosan-β-cyclodextrin nanoparticles	MD: 225.9 nm; PDI: 0.185; ZP: 19.3 mV	The nanoparticles provided better acaricidal activity, higher repellency, and reduced oviposition in *Tetranychus urticae*, compared to non-encapsulated compounds. Similarly, the nanoparticles showed better insecticidal activity and greater residual effects on the development of *Helicoverpa armigera*, compared to the non-encapsulated control.	[66]

MD: mean diameter; PDI: polydispersity index; ZP: zeta potential.

2.2 Green nanomaterials in innovative biosensor application

Nano(bio)sensors exploit the unique properties of nanomaterials for diverse purposes: i) the immobilisation of the bioreceptor on a transducer; ii) the functionalisation of the bioreceptor for the configuration of optical or electrochemical transduction; iii) the miniaturisation and integration of biocomponents, transduction systems, electronics and microfluidics in complex architectures. Once projected, these nanosystems show potential for agricultural applications, being able to evaluate crop maturity and health status, detect and tune the amount of fertilisers and pesticides spread, and sense soil humidity to tailor irrigation avoiding water misuse.

In this context, (bio)sensor technology is one of many branches which could profit from the boost in the manufacture of innovative and sustainable products provided by green strategies and resulting in an increased number of biosensors developed through the exploitation of green nanomaterials. Gold and silver nanoparticles synthesised using the flower extract of *Rosa damascena* have been used as reducing and stabilizing agents to modify glassy carbon electrodes, showing an increased electronic transmission rate between the modified electrode and $[Fe(CN)_6]^{3-/4-}$ [86].

Allium cepa L. (onion) extracts have been used to biosynthesise silver nanoparticles for the modification of a three-electrode cell [87]. The high phenol content in the onion extract with strong properties is exploited as reducing and stabilizing agent for the synthesis of Ag nanoparticles. The authors highlighted the ability of the green synthesized silver nanoparticles to reduce charge transfer resistance, as confirmed by electrochemical impedance spectroscopy. Their effect in the detection of ascorbic acid using voltammetric methods was also explored, showing a good linear range (0.4 - 450 µM) with 0.1 µM of detection limit.

Green AgNPs have also been synthesized by Sebastian et al., [88] by a simple approach based on bark extract of Moringa oleifera (MO). This bark contains a large amount of benzyl glucosinolate, which influences the agglomeration of AgNPs, through the formation of a covalent bond between its sulfur group and the nanoparticles (AgNP-S). This leads to aggregation of AgNPs and consequently to complex formation with Cu(II) ions through the hydroxyl group of benzyl glucosinolate (Cu(II)-O). This mechanism was exploited for the development of both optical and electrochemical sensors for Cu(II) ions sensing, by colorimetric absorption for the first, and by differential pulse voltammetry using AgNP-MO-modified platinum electrode for the latter. Both sensors allowed a Cu(II) ions detection in the concentration range from 10 to 90 µM, with a detection limit of 0.530 µM.

Green gold and silver nanoparticles have been synthesised using quercetin as reducing agent to fabricate a third generation lactose biosensor based on cellobiose dehydrogenase from *Trametes* *villosa*, with increased electroactive areas and electronic transfer rate constants [89]. Indeed, the resulting biosensor showed very efficient direct electron transfer, a detection limit for lactose of 3.5 mM, a large linear range from 10 to 300 mM, a high sensitivity (5.4 $mAmM^{-1}$ cm^{-2}) and long-term stability.

Alzahrani and co-workers studied the detection of mercury II ions using green silver nanoparticles as colorimetric probe [90]. Onion extract was used for the synthesis of AgNPs without impurities, as confirmed by the results of the EDAX analysis. Hg^{2+} was sensitively and selectively detected by surface plasmon resonance in both standard solutions and real water samples (tap water and groundwater) with an RSD of less than 6% and a recovery of more than 92%.

A novel biosynthesis approach for the preparation of Au nanoparticles has been reported based on green alga *Pithophora oedogonia* as reducing agent. The biosynthesized Au nanoparticles, with an average diameter of 33 nm, were then used for the modification of screen-printed electrodes in the amperometric detection of trace amount of carbendazim [91]. A linear detection range was found between 0.05-25 µM, with detection limit 2.9 nM. Satisfactory results were obtained in soil samples with recovery values of ca. 100%.

Quercus glauca leaves extract was used to synthesize platinum nanoparticles for the detection of environmental and human toxic hydrazine [92]. The synthesized green PtNPs were exploited for the modification of a glassy carbon electrode, showing a good electrocatalytic activity to the electro-oxidation of hydrazine (Figure 3A). This demonstrated the capability of the green synthesized PtNPs as excellent electron mediators to improve the electrochemical properties for the oxidation of hydrazine, which was amperometrically detected within a wide linear range (0.01-283 µM), low detection limit (7 nM), and good sensitivity (1.704 $\mu A/\mu M/cm^2$).

Palladium nanoparticles with controlled size have been produced by green synthesis for biosensor application, exploiting different sources including *Cinnamomum camphora* leaf extract [93], *Cinnamomum zeylanicum* bark extract [94], and *Gardenia jasminoides Ellis* crude extract [95].

As an example, *Sargassum* alga extract was used as an alternative route to synthesise palladium nanoparticles to modify carbon ionic liquid electrode for the amperometric determination of H_2O_2 [96]. The authors were able to obtain well dispersed nanoparticles, with an average mean size of 5 nm, and fairly stable up to 5 months. The resulted biosensor exhibited a high selectivity and sensitivity (284.35 μAmM^{-1} cm^{-2}), wide linear range (from 5 µM to 15 mM), and good stability.

Carbon dots synthesis can exploit chocolate [97], bamboo leaves [98], rose-core radish [99], pork [100], and aloe as carbon source [101]. Water-soluble nitrogen-doped carbon dots were obtained via one-pot hydrothermal carbonization of natural peach gum polysaccharide and ethylenediamine mixture, with remarkably enhanced quantum yield (28.46%) compared to undoped carbon dots (5.31%) [102]. Moreover, the authors underlined characteristics such as highly stable fluorescence against ionic strength variation and pH change, as well as low cytotoxicity. The implemented nitrogen-doped carbon dots were able of Au^{3+} ions quantification in water with detection limit of 6.4×10^{-8} M, by means of gradual decline of their emission intensity at 445 nm (λ_{ex} = 370 nm) in the presence of varying ions concentrations in the range of 0-50 μM.

Carbon nanodots with quantum yield of about ~86% have been synthesized by hydrothermal method from citric acid and diethylenetriamine, exploiting quercetin and its fluorescent metal-ion complex (QCT-Al^{3+}) efficiently coordinated on the surface of the nanodots [103]. Indeed, carbon nanodots emission at 429 nm completely overlapped with QCT-Al^{3+} absorption peak at 428 nm, allowing for the design of a Forster resonance energy transfer (FRET) sensor for Al^{3+}. A linear calibration of F_{429}/F_{481} versus aluminium ion concentration was obtained within 1–20 and 20–60 μM, with a detection limit of 558 nM.

Shariati et al., described the green synthesis of carbon dots from *cedrus* plant, to develop an optical sensor for the determination of propranolol. In detail, the authors exploited a layer of molecular imprinted polymers (MIPs) coated with the green synthesized CDs [104]. The sensor response was linear in the range of 0.8- 65 nmolL^{-1}, with a detection limit of 0.2 nmolL^{-1}, and it showed various advantages in terms of rapid response, high sensitivity, selectivity, and cost-effectiveness.

The green synthesis of carbon dots functionalized silver nanoparticles (CDs-AgNPs) using *lycii Fructus* was also successfully applied for development of a colorimetric sensor to monitor phoxin in environment and fruit samples [105]. In particular, CDs-AgNPs aggregation was observed by colour change from yellow to red, in the presence of phoxim. This mechanism allowed for phoxim detection with a low detection limit (0.04 μM), as well as high sensitivity, selectivity, and good recovery values ranging from 87% to 110.0% (RSD of 6.0%).

Graphene and graphene-based composites for (bio)sensor applications have been produced by green routes using *cocos nucifera* L. (coconut water) [106], *Bacillus marisflavi* biomass [107], and rose water as reducing agent [108]. In different studies, green synthesis has been used to decorate graphene with nanomaterials. As an example, a simple one-pot hydrothermal method using gallic acid as the reducing agent was exploited to decorate reduced graphene oxide sheets with gold nanoparticles, for the design of a nonenzymatic sensor to detect H_2O_2 [109]. This sensor provided a linear response in a concentration range of 0.05-5 mM (R=0.999), and a high sensitivity of 255 μA cm^{-2} mM^{-1}.

Reduced graphene oxide/gold nanoparticles (rGo/AuNPs) were produced by green synthesis to develop an electrochemical biosensor for detection of tryptophan, using *E. tereticornis* leave extract as an environmentally friendly reducing reagent [110]. This composite was exploited as electroactive substrate on the surface of screen-printed electrodes to measure tryptophan oxidation with low detection limit (0.39 μmol/L), linear range from 0.5 to 500 μmol/L, good reproducibility, high sensitivity, and selectivity (Figure 3B). The determination of tryptophan in serum, saliva, and plasma samples was also reported with recoveries for the spiked concentrations from 94 to 107%.

Zinc oxide and copper oxide nanoparticles were successfully synthesized following an ecological approach by using *Camellia japonica* leaf extract as inductive and stabilizing agent [111]. The synthesized metal oxide nanoparticles were applied for the colorimetric determination of metals ions such as Ag^+ and Li^+ in marine water (Figure 3C).

Other composites have been also obtained by using green nanomaterials, as the palladium/poly(3,4-ethylenedioxythiophene) (PEDOT) nanocomposite produced to modify glassy carbon electrode to detect H_20_2 [112]. The palladium was produced with an average diameter of 4.5 nm by green method without surfactants or templates using H_2PdCl_4. The electrochemical response of Pd/PEDOT to H_20_2 demonstrated a good sensitivity, good repeatability and stability, and a low limit of detection (2.84 μM) in the range of 2.5×10^{-3} - 1 mM.

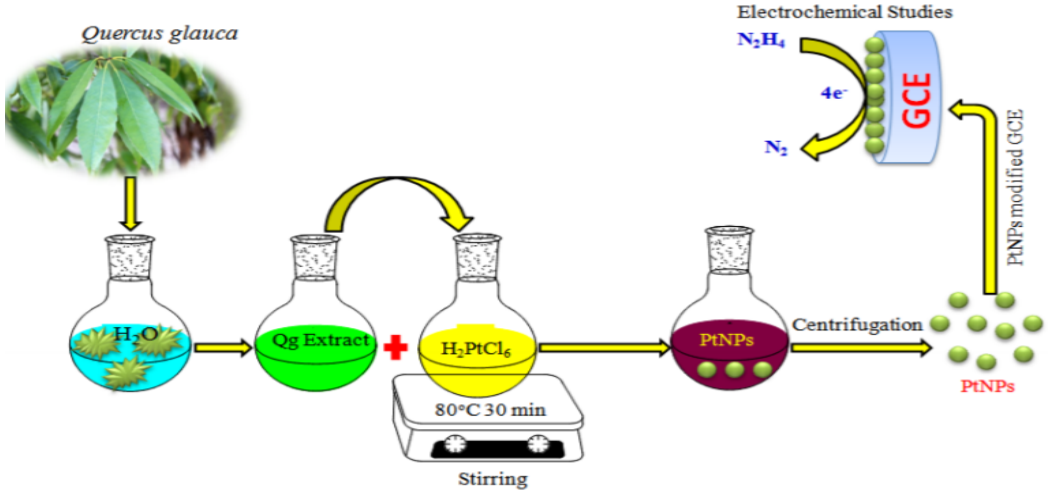
Table 3 reports the diverse biosensors developed by the use of nanomaterials produced via green synthesis. However, to the best of our knowledge, few examples of biosensors exploiting green nanomaterials have been described in literature, being nanomaterial's green synthesis an emerging field.

Table 3. Biosensors developed by the use of nanomaterials produced via green synthesis.

Nanomaterial	Synthesis	Biosensing configuration	Transduction	Target analyte	Linear range	LOD	Ref.
Silver nanoparticles for three-electrode cell modification	*Allium cepa* L. (onion) extracts	AgNPs carbon paste electrode	Square wave voltammetry	ascorbic acid	0.4-450 µM	0.1 µM	[87]
Silver nanoparticles	Moringa oleifera	AgNP-MO-modified platinum	Colorimetric absorption and differential pulse voltammetry	Cu(II)	10-90 µM	0.530 µM	[88]
Gold and silver nanoparticles	Quercetin	Cellobiose dehydrogenase immobilised on AuNPs and AgNPs modified graphite electrode	Amperometry in a flow injection analysis	Lactose	10-300 mM	3.5 mM	[89]
Silver nanoparticles	Onion extract	In solution	Surface plasmon resonance	Hg2+	-	-	[90]
Gold nanoparticles	*Pithophora oedogonia* alga extract	AuNPS modified screen printed electrode	Amperometry	carbendazim	0.05-25 µM	2.9 nM	[91]
Platinum nanoparticles	*Quercus glauca* leaves extract	PtNPS modified glassy carbon electrode	Amperometry	Hydrazine	0.01-283 µM	7 nM	[92]
Palladium nanoparticles	*Sargassum* alga extract	Pd nanoparticle-modified carbon ionic liquid electrode	Amperometry	Hydrogen peroxide	5 µM-15 mM	2.84 µM	[96]
Carbon dots	Peach gum polysaccharide and ethylenediamine mixture	In solution	Fluorescence	Au^{3+}	0-50 µM	6.4×10^{-8} M	[102]
Carbon nanodots	Citric acid, diethylenetriamine, and quercetin	CNDs/QCT immobilised on filter paper	Fluorescence resonance energy transfer	Al^{3+}	1-20 µM and 20-60 µM	558 nM	[103]
Carbon dots	*Cedrus* plant	MIPs coated with CDs	Fluorescence	Propranolol	0.8- 65 nM	0.2 nM	[104]

Carbon dots functionalized silver nanoparticles	*Lycii Fructus*	In solution	Spectrophotometer	Phoxim	0.1-100 µM	0.04 µM	[105]
Reduced graphene oxide/gold nanoparticles	*E. tereticornis leave extract*	rGO/AuNPs modified screen printed electrode	Response surface methodology	Tryptophan	0.5-500 µM	0.39 µM	[110]
Zinc oxide/copper oxide nanoparticles	*Camellia japonica leaf extract*	In solution	UV-Vis	Ag^+ and Li^+	10-100 µM	-	[111]
Palladium/poly(3,4-ethylenedioxythiophene) (PEDOT) nanocomposite	Green method without surfactants or templates using H_2PdCl_4	Pd/PEDOT nanospheres modified glassy carbon electrode	Amperometry	Hydrogen peroxide	2.5×10^{-3} - 1 mM	2.84 µM	[112]

A


Quercus glauca
Electrochemical Studies
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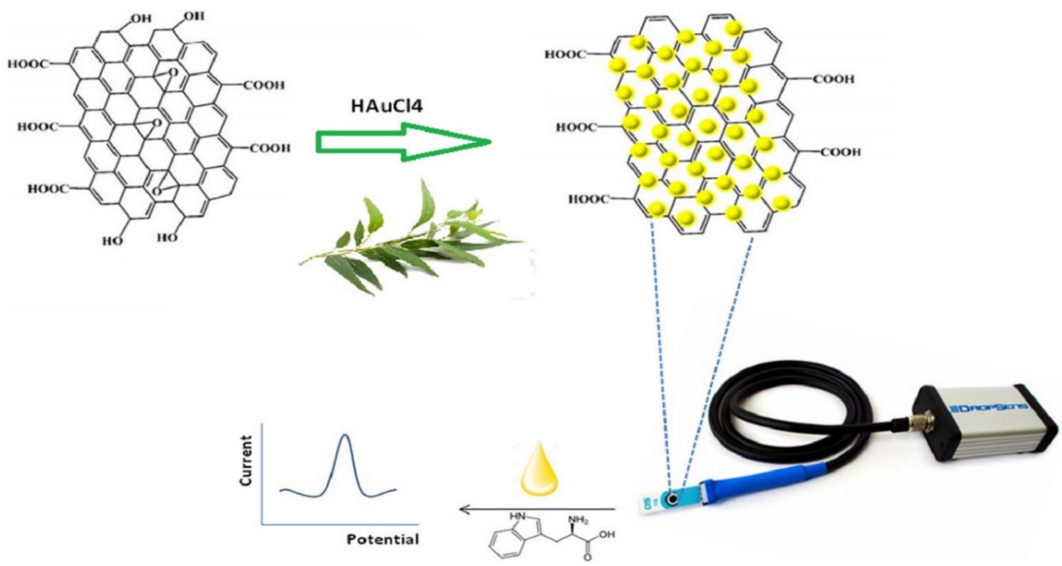
Stirring

B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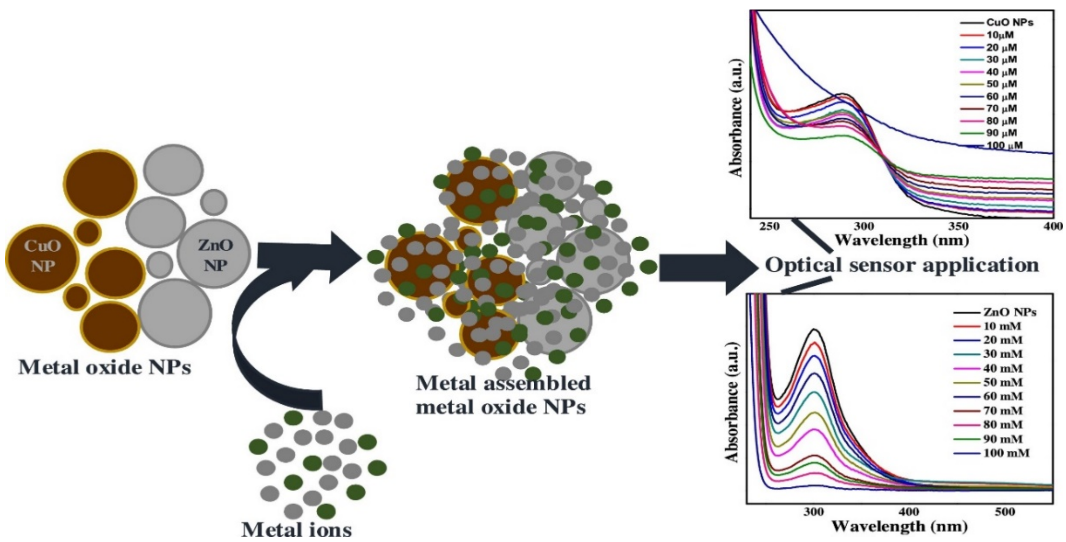
CuO NP
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Optical sensor application
Absorbance (a.u.)
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Absorbance (a.u.)
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ZnO NPs
10 mM
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60 mM
70 mM
80 mM
90 mM
100 mM
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400
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Figure 3. A) Synthesis of PtNPs and application for the electrochemical detection of hydrazine. Reprinted with permission from R. Karthik, R. Sasikumar, S.M. Chen, M. Govindasamy, J. Vinoth Kumar, V. Muthuraj, Green synthesis of platinum nanoparticles using *Quercus glauca* extract and its electrochemical oxidation of hydrazine in water samples, Int. J. Electrochem. Sci. 11 8245–8255. Copyright (2016) Published by ESG [92]. B) Green synthesis of reduced graphene oxide/gold nanoparticles for electrochemical sensing application. Reprinted with permission from S. Nazarpour, R. Hajian, M.H. Sabzvari, A Novel Nanocomposite Electrochemical Sensor based on Green Synthesis of Reduced Graphene Oxide/Gold Nanoparticles Modified Screen Printed Electrode for Determination of Tryptophan using Response Surface methodology Approach, Microchem. J. 104634. Copyright (2020) Elsevier journals [110]. C) Schematic representation of metal ions induced assembly of metal oxide NPs for optical sensing application. Reprinted with permission from M. Maruthupandy, Y. Zuo, J.S. Chen, J.M. Song, H.L. Niu, C.J. Mao, S.Y. Zhang, Y.H. Shen, Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) via biological template and their optical sensor applications, Appl. Surf. Sci. 397 167–174. Copyright (2017) Elsevier journals [111].

3. Gaps, obstacles, and trends of green nanofertilisers, nanopesticides, and nano(bio)sensors

According to a recent report released by StatNano, about 13,046 published patents related to nanotechnology were registered at the United States Patent and Trademark Office (USPTO) and the European Patent Office (EPO) in 2018. The United States is the country with the greatest number of patents registered in the USPTO database, while Japan occupies the top position in the EPO database, followed by the United States [113]. A search was performed for patents in the World Intellectual Property Organization database, registered from 1976 up to the present time, using four sets of word combinations: i) nano* AND agriculture; ii) nano* AND pesticides AND agriculture; iii) nano* AND fertilizers; and iv) nano* AND pest control AND agriculture. Around 2,300 patents matching these criteria were found in the database. In a second search, around 1,500 patents were found using the following two sets of word combinations: i) biogenic nanoparticles AND agriculture AND crop protection; and ii) green nanoparticles AND agriculture AND crop protection. It should be noted that while most of these patents speculate on the use of the developed nanoparticle and/or nanosystem in agriculture, there no tests and/or evidence of the use of the systems in the field are reported in the patents.

The patent's analysis indicates that the application of nanotechnology in agriculture has increased over the years. However, the application of green nanotechnology to the agricultural area is in its infancy, especially when compared to its development in other areas, especially medicine [5].

Nano-based formulations have both the potential to address challenges related to agriculture, as well as to contribute to its sustainable development. However, there are significant limitations and gaps that must be addressed [114,115]. By definition, all nanomaterials, independently of their methods of production, hence also green nanomaterials, exploit new properties not shown by their macro counterparts, and these characteristics have shown to be extremely useful in providing innovative solutions, outlined by their adaptability, responsiveness and possibility of functionalization. However, it is exactly these characteristics which may induce new interactions with the human system and the environment [116], and require adequate toxicological tests and life cycle assessments.

The present legislation is still in an early stage of development [117], and several obstacles hinder the regulation of nanomaterials in the agricultural sector, one of the most important one being the lack of a common international definition of nanomaterials. This void needs to be addressed in order to avoid products falling into regulatory gaps [105-107]. The lack of appropriate analytical techniques able to identify, characterize and quantify nanomaterials in complex mixtures (such as agricultural samples), without previous isolation of the nanomaterial of interest further hampers the formulation of sensible regulations. Efforts should be made to increase the sensitivity of techniques used to determine size, characteristics, and concentration of nanomaterials [118,119]. Although many publications have shown the potential of nano-based formulations, their utilization in the agricultural field remains unclear and currently there are only few nanotechnology-based products available on the market [120]. This may also be due to the reluctance of publicly addressing the use of nanotechnology in the agrifood sector. Public acceptance and awareness are key factors influencing the success of innovative applications, and while consumers are more willing to accept nanotechnological applications in the medical sector, the prefix "nano" in combination with agriculture and food seems to be the opposite of the trendy terms of "natural", "organic" and "eco-friendly". It is the responsibility of the scientific community to share knowledge, and that of the industries to foster transparency, in order to contribute to a climate of confidence, reassuring the public, that their safety and their interests are looked after.

Economic issues also hinder wider marketing of nanocompounds, in particular of nanopesticides. Compared to conventional formulations, higher initial investments are required to develop nano-based formulations, which can only be compensated by the widespread use of these products in the field. In addition, the registration of any new active substance is expensive [121]. Lower production costs of green nanoformulations could partially address these issues. Even though industrial scale production of green nanomaterials has not been performed yet, it is feasible to assume that lack of chemical compounds, as well as very low energy requirements, will prove to reduce costs. Another option to increase the cost-effectiveness of biosynthesized nanomaterials is through the utilization of recyclable waste materials, which would introduce a further added sustainable value directly targeting SDG 12 [122].

4. Conclusions

Nanofertilisers, nanopesticides, and nano(bio)sensors applied to agriculture have the potential to disruptively address many of the great challenges that mankind will be facing in the coming decades. This is particularly true for green nanomaterials since their production strives to minimize the impact on ecosystems, increasing their efficiency and sustainability. However, a deeper and more comprehensive understanding of the potential benefits and risks of nanotechnology must be acquired, in order to be able to address consumers' concerns. The agri-industry is a trillion-dollar business and the adoption of environmentally-friendly nanotechnological approaches, if correctly regulated and applied, can play a key role in the achievement of the SDGs.

Acknowledgments

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Oggetto: Re: TRAC Special issue "Biosensors for Agricultural and Food Safety"
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Data: 16/07/2019, 11:05
A: Viviana Scognamiglio <viviana.scognamiglio@ic.cnr.it>
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Excellent Vibian, will add into the list.

Kind regards

Damia

El 16/07/19 a las 10:26, Viviana Scognamiglio escribió:

> Dear Prof. Barcelò,
>
> I would like to thank you very much for your consideration in kindly inviting me for the submission of a review on your special issue.
>
> In attached file please you can find our tentative draft of a proposal review, hoping you will find suitable for the issue.
>
> Thanks again and warm regards,
>
> Viviana
>
> --
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>
> Hello,
>
> Together with Prof Jianfeng Ping, from Zhejiang University. Recently, we are organizing a

Virtual Special Issue for **TrAC-Trends in Analytical Chemistry** on the topic of "**Biosensors for Agricultural and Food Safety**".

Agricultural and food safety is an increasingly important public health issue for both consumer and food industry. Within the past few decades, the agriculture and food industry have experienced an incredible growth both in terms of the amount of foodstuff produced and sold. The growing production of foodstuff and the ever-present threat of agricultural and food contamination have forced the people to pursue simple, rapid, and cost-effective analytical approaches for harmful residues in agricultural products to ensure the health of consumer. To date, numerous work have been performed in the development of analytical methods for qualitative/quantitative detection of harmful residues in agricultural products. With the rapid development of nanotechnology and biological technology, biosensing techniques integrate with new materials and devices have shown great potential in agricultural and food industry.

To overview the recent progress on biosensors for the detection of harmful residues in agricultural products, this Special Issue of Trends in Analytical Chemistry (TrAC) entitled “Biosensors for Agricultural and Food Safety” will provide a platform and an opportunity to promote mutual cooperation, information dissemination and exchange among researchers. The Special Issue features review articles from a wide range of scientists active in biosensing strategies in agricultural and food industry, and highlights new concepts for agricultural and food safety related biosensors that are on the basis of novel biosensing technology and new materials design, which would promote development for quick and effective determination of harmful residues in this field.

Given your expertise and contributions to this field, it would be really nice to have a review from your group. If you can contribute a manuscript, please let us know before the edn of August 2019. The deadline for submission is Dec 30, 2019. The issue will be published on July 30, 2020.

Best wishes,

Jianfeng

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