

REVIEW

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Advancing the impact of plant biostimulants to sustainable agriculture through nanotechnologies

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Abstract

Nanomaterials and plant biostimulants are attracting significant attention for their potential applications in the agri-food sector. These efforts have mostly focused on the independent applicability of these two emerging fields to achieve improved agricultural outcomes. However, an even larger impact can be created if the emerging fields of nanotechnology and biostimulant technology are combined. This transdisciplinary review combines two diverse fields to highlight the potential role of nanotechnologies in advancing the impact of plant biostimulants for the sustainable production of high-quality food. The review first explains the key concepts of nanotechnology in a tutorial style to offer the agri-food research community an improved understanding of the potential of nanotechnology. The review then dives deeply into plant biostimulants which are substances or microorganisms or complex mixtures capable of improving plant nutrition and growth, directly influencing the aspects relevant to food quality, safety, and security. The review presents a convincing case that combining nanomaterials with biostimulants provides a promising sustainable answer against food insecurity for the near future. Sustainability is central to our discussions, which is exemplified by cases, such as the production of plant biostimulants from waste material, thus contributing to a circular economy. The review also identifies promising avenues to utilize nanotechnology for improving the value of biostimulants. Strategies such as nanoencapsulation are proposed to produce nano-biostimulants that could act synergistically to enhance food quality while offering a solution to the increasing challenge of food production while respecting the environment.

Keywords Biostimulants, Plant growth, Nanotechnology, Nanoparticles, Fertility

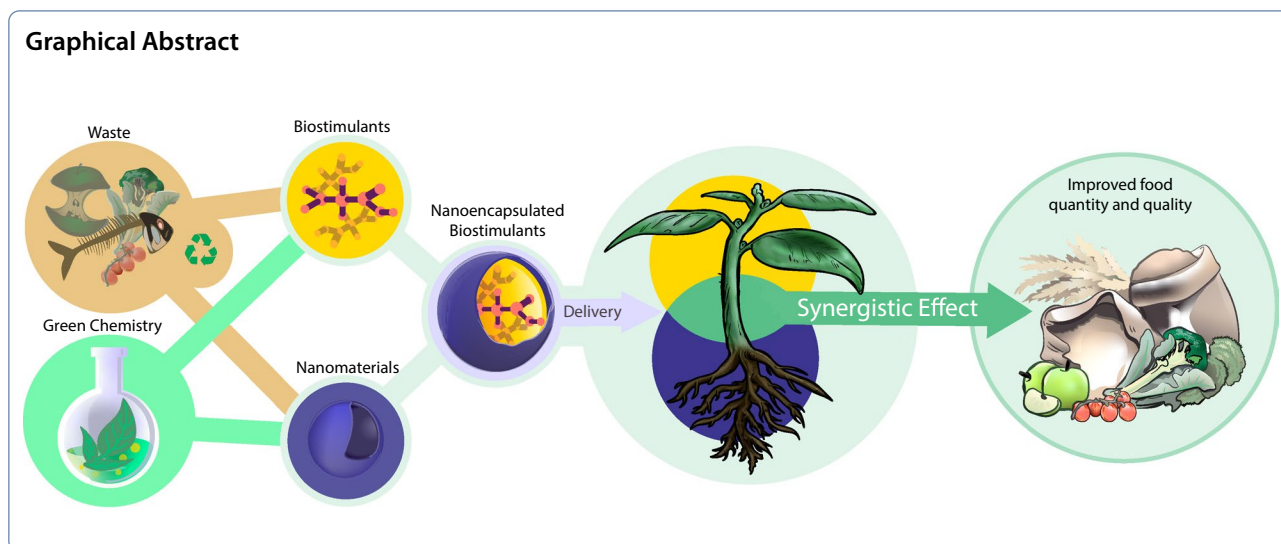
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Introduction

In this period of deep changes in the world and society, the agriculture sector faces numerous new challenges while aiming at sustainably feeding the increasing global human population. The world's population, estimated to be 8.031 billion in 2023, is projected to continue growing, reaching around 9.7 billion in 2050 and rising further to approximately eleven billion by the end of the century. The continuous increase in global population, coupled with the expected rise in global prosperity, will place further pressure on natural resources and agricultural ecosystems [1] that are already on the verge of their tipping capacity. Climate change, which is intimately associated with greenhouse gas production from human activity, represents another major threat to food security and sustainable land management. Burning fossil fuels has increased the concentration of atmospheric carbon dioxide (CO₂) reaching a concentration of 417 ppm in 2021, a considerable increase if compared with a concentration of 378 ppm in 2005. Carbon dioxide combined with other gases increases the temperature of the Earth's surface by warming the lower layer of the atmosphere [2]. The greenhouse effect is exacerbated by a change in the anthropogenic land coverage, leading to biophysical variation of the energy surface of our planet, causing unpredictable and extreme climate events [3, 4]. This alteration in the landscape and atmosphere of our planet continues to modify many ecological interactions, including the carbon cycle [5, 6].

These emerging factors add a significant burden to the existing challenges in the agricultural sector which already faces the issues around global food chains, market competition, industrial processes, and the need for increasing productivity. Furthermore, the sector needs

to balance between profitable economic outcomes and reduced environmental impacts. A new sustainable agriculture must be able to feed a growing population, provide a livelihood for farmers, and protect the environment. These represent the crucial challenges to be addressed for providing a sustainable food security, as recently reported by Organization for Economic Cooperation and Development (OECD) [7].

In this scenario, innovation can play a strategic role to adapt to emerging changes and support modern agriculture [8]. This could be achieved, for instance, by developing innovative tools, technologies and products, and in such scenarios, nanotechnology might represent the way forward for improved and sustainable agricultural production [9, 10], providing a plausible answer to the problems of modern agriculture in line with the European strategies. In 2020, the European Commission (EC) proposed the "Farm to Fork Strategy" as the heart of the wider Green Deal strategy. In this policy document, by 2030, Europe aims for a 50% reduction in the use of chemical pesticides, a reduction in nutrient losses of at least 50%, and a reduction in the use of fertilizer by at least 20%. In addition, the EC strategy plans to increase organic cultivation of agricultural land to 25%.

One of the steps made in the direction of improving modern agriculture sustainability was the introduction of plant biostimulants in the crop cultivation. These substances, as further explained later, allow to reduce the chemical inputs in the crop production, by enhancing the plants' physiological and metabolic responses to biotic and abiotic stress. These compounds could be produced from different sources, even waste material, and their strength lies in high efficacy with a very small amount for treatment, but their weakness is the short time of actions

and the high environmental degradability due to their composition. To face the modern agriculture challenges the biostimulants alone could be not enough. Nanosystems might represent a way to overcome current biostimulant limitations.

Nanosystems and biostimulants together can make a credible contribution to fulfilling sustainable agriculture objectives. Both of these tools have the potential to positively influence the physiology and metabolism in plants, making these two approaches of strategic importance to sustain crop productivity and support plant metabolism against climate change [11–14]. An unprecedented possibility is to combine these two technologies, paving the way for a new synergistic approach to address the challenges of modern agriculture. Merging biostimulants and nanotechnology opens new possibilities for creating a more effective field strategy with reduced environmental impacts while maintaining product quality and quantity, still being profitable for the farmers.

This review aims to present the state of the art in the possibilities of producing nanotechnology-enhanced biostimulants through nanoencapsulation and developing nanocarriers. Nanotechnology can be deployed as an effective tool for the design, development, and delivery of a new generation of biostimulants, effectively supporting the growth and development of crop plants and their yielding potential. For completeness, the review also briefly captures upon the key aspects of nanotechnologies and their other potential applications in agriculture.

For the agricultural sector, scientific studies since 1999 have highlighted the potential application and improvement that nanotechnology could give to food and agrosystems [15, 16]. The interest in this technology is growing in the scientific community over the last few years. Searching the terms “nano*” and “agri*” in the Web

of Science platform (<https://www.webofscience.com/>), the last decade (2012–2022) has seen the publication of 32,960 documents that deal with the potential amalgamation of nanotechnology with agriculture (Fig. 1). On a comparative note, a recent publication accounted for 1088 articles focusing on biostimulants published over the last 20 years [17].

Nevertheless, the data available from the literature also show that this technology has found more applications in the food industry than in agriculture [15]. For instance, the application of nanotechnology in the food industry has been proposed to enhance food safety by employing nanosensors [18], or through combining nanotechnology with other omics-based approaches to facilitate the discovery of new food safety biomarkers [19], or through the development of more sustainable smart food packaging solutions enabled with advanced nanotechnologies [20]. On the other hand, the nano-techniques employed in the agriculture sector are mainly focused on developing specific desirable characteristics, for example, to increase the effectiveness of materials or molecules (e.g., by increasing their solubility or stability), to achieve their controlled release, and for their targeted delivery [15]. These characteristics may find use in specific applications, such as nano-biosensors, and nanoparticles (NPs) for soil remediation, as nanofertilizers and as nanopesticides [9].

Nanotechnologies and their applications in agriculture

Nanotechnologies have greatly improved modern life with a credible positive impact on medicine, the environment, industrial processing, and sensing [1]. The application of nanotechnology in agriculture could significantly improve the efficiency of agricultural inputs and therefore offers a significant way forward to maintain

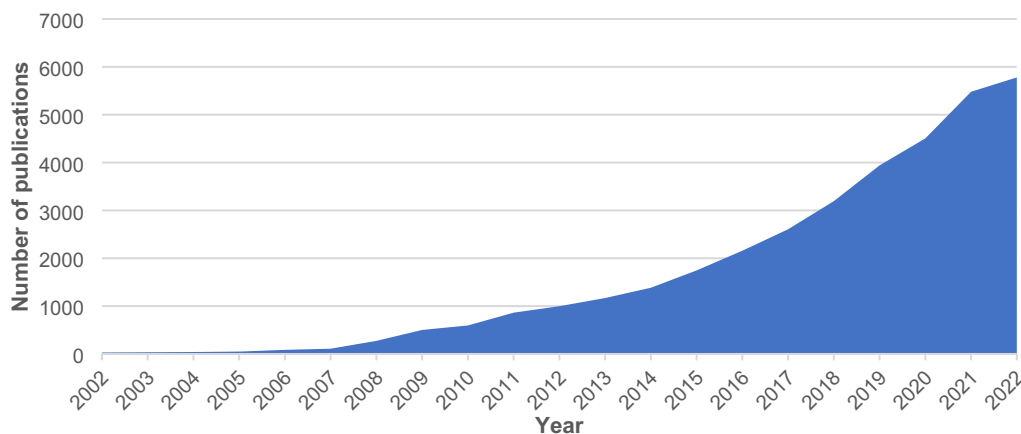


Fig. 1 Publications reported over the past 20 years while searching for the keywords “nano*” and “agri*” in the Web of Science database (<https://www.webofscience.com/>)

agroecosystems' sustainable development. Nanotechnology today offers several potential benefits for agriculture, including increasing agricultural production, inhibiting plant pathogens, and reducing the inadvertent release of chemicals into the environment. Different applications of nanotechnology have been proposed and applied, including nanosensors [21], nanofertilizers [22, 23], and nanopesticides [24, 25]. In the following sections, we touch upon these aspects, starting with a brief description of nanomaterials for non-experts in the field, followed by discussing some of the potential applications of nanotechnology in agriculture, and last but not the least, also outlining the potential risks of nanotechnology for the agriculture and the environment that we must consider while developing nano-products for the agricultural sector.

Nanoparticles (NPs) and nanomaterials (NMs)

According to the United States National Nanotechnology Initiative (NNI), nanotechnology is a field of science, engineering, and technology characterized by the operation of materials at nanoscale size (1–100 nm) [26]. The properties of nanoparticles (NPs) vary according to their size: they can be considered colloidal particles with an extremely high ratio of surface-to-volume and high specific surface area. Among various factors, this high surface area of NPs is the key factor that leads to their excellent chemical, physical, biological, mechanical, and electronic properties [27], and is responsible for the major buzz created by nanotechnology over the past few decades. Of course, in addition to their small size that leads to the high surface area, NPs may also be classified by their morphology, chemical composition and surface characteristics, each of them producing a unique property [28].

The terms NPs and nanomaterials (NMs) are often used interchangeably. But for the purpose of this review, let us consider NPs as particles that have at least one of their dimensions below 100 nm, while NMs also encompass multi-component systems that have at least one of their components as NPs. For instance, a nanowire that is less than 100 nm thick, but several microns long can be classified both as an NP and an NM, as it is a single-component system. In another example, a hollow nanosphere (a nanocapsule) that is less than 100 nm in diameter and is filled with a drug or a biostimulant may be considered an NM, as it illustrates a multi-component system with both NP and a drug/biostimulant component present. As such, a variety of NPs and NMs can be produced, and these may provide immense benefits in agricultural and plant management, since their applications may result in enhanced efficacy, reduced input, and lower eco-toxicity,

in comparison with conventional products and technologies [29].

The production of NPs and NMs for various applications has rapidly increased over the past decade. However, considering the vast activity in this area, it is challenging to obtain comprehensive data on their production. Nevertheless, their worldwide production is constantly increasing, as reflected by an estimated production of ~500 tons of nanosilver in 2008 globally [30], and ~38,000 tons of nano-titanium dioxide (TiO₂) in 2017 in the USA alone [31]. Europe produced the highest amount of nano-silica (SiO₂) in 2016 with an annual production of 55,000 tons [31] and total production and importation of NMs of around 1,615,000 tons [32]. These are only some of the most prominent examples of a large repertoire of innovative materials produced by nanotechnology, a highly cross-disciplinary technology with wide application possibilities in several fields [33].

Concerning the production of NPs and NMs, two broad approaches are employed, including a top-down and a bottom-up approach [34]. As illustrated in Fig. 2a, the top-down approach corresponds to the manufacturing of micro and nanostructures from a larger material (typically referred to as 'bulk' material) by employing a subtractive way via sculpting or etching bulk materials into desired shapes and sizes. The top-down strategy predominantly includes physical methods including photolithography, soft lithography, etching, and ball milling [35]. One benefit of some of these highly controlled top-down approaches, such as lithography is that the materials produced have easily interchangeable variables and the possibility to create complex shapes; however, such top-down methods can be difficult to scale to larger production due to considerable process development

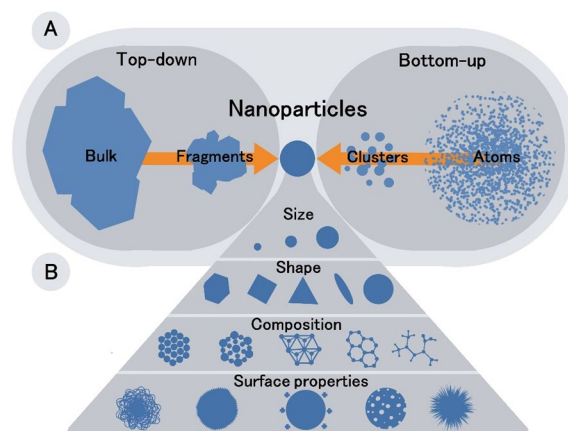


Fig. 2 Graphical representation of **A** two key strategies employed for nanoparticle synthesis, and **B** a summary of factors that can influence the properties and applicability of nanoparticles

requirement [34]. On the other hand, some other top-down approaches such as ball milling are scalable; however, these methods do not provide good control over the size and shape of NPs due to the randomness associated with these ball milling processes. Conversely, the bottom-up strategies, as the name suggests and illustrated in Fig. 2a, rely on starting from the monomeric atomic units that are combined in a controllable fashion to obtain nanoscale materials. For instance, bottom-up strategies may take advantage of the self-assembly of amphiphilic lipids or macromolecules driven by the influence of the chemical environment (pH, solvent, temperature, and salt). These self-assembly methods can be easily scaled up, and control over particle internal structure can be obtained. Other examples of bottom-up synthesis include the production of metal NPs, like Au and Ag by employing metal ions, their appropriate reducing agents, and generally a surfactant that acts as a capping agent during the synthesis to control the size and morphology of the NPs [36]. Most of the chemical synthesis approaches represent examples of bottom-up synthesis approaches. These bottom-up synthesis approaches can be highly susceptible to processing conditions and may sometime impose limitations around scalable synthesis, as particles obtained via colloidal synthesis may aggregate beyond a certain population density in the reaction media. Certain physical methods can also be classified under bottom-up approaches, such as those involving physical vapour deposition (PVD) strategies [37]. As an illustration, bulk gold can be melted at relatively low temperatures under a vacuum and evaporated under control conditions to deposit on a substrate. This allows the production of nanostructured surfaces, which can then be used for a variety of applications. A similar strategy is employed during chemical vapour deposition (CVD) methods; however, a chemical vapour is used instead of a physical vapour [38]. A vast majority of electronic devices with nanoscale components are produced by these types of methods, as the methods offer a high degree of reproducible outcomes, which are critical for such applications.

In addition to the above physical and chemical routes of NP synthesis, researchers have developed nanoparticle synthesis with biological approaches to minimize the use of toxic chemicals. This green synthesis represents an innovative approach that includes the use of plant extracts, microorganisms, fungi and biological molecules such as enzymes, proteins, sugars and lipids as a new platform for the production of a range of NPs [39, 40]. Biological approaches for the synthesis of NPs can also be exemplified by both top-down and bottom-up strategies. The bottom-up biological synthesis strategies are like those employed during the chemical synthesis of NPs, with a variation that at least one of the chemicals

employed in the chemical synthesis method (e.g., a reducing or hydrolyzing agent) is replaced with a biological agent (e.g., a microorganism, plant extract or a biomolecule) [41]. The top-down biological synthesis strategies are akin to the bioleaching processes commercially employed by certain mining industries to leach high-value metals such as copper from their ores. However, in the case of NMs synthesis, a carefully chosen microorganism can selectively leach out high-value NPs from their original source. This approach has been illustrated, for instance, for producing highly crystalline silica from amorphous silica in rice husk [42], and selective isolation of crystalline silica from zircon sand [43]. The process offers significant value creation opportunities, as rice husk is an agro-industrial by-product, while the quality of zircon sand which is a commercially important refractory material, improves after this bioleaching process.

Depending upon the chosen synthesis route, several important properties of NPs and NMs can be controlled. As illustrated in Fig. 2b, these include size, morphology, composition, and surface characteristics. The importance of the size of NPs on their properties has already been discussed above. Similarly, the shape of NPs also affects their behaviour, e.g., how they absorb light [44], or how they interact with the biological world [45]. The composition, of course, decides their suitability for the specific application. For instance, a low-cost environmentally compatible NM, such as silica would be more appropriate for an agricultural application, while an expensive NM, such as gold nanoparticles may be more suitable for biomedical applications. Last, but not least, the surface characteristics of NPs are also one of the most crucial factors that dictate their final properties, as NMs and NPs interact with their surrounding through the exposed surface. In this context, irrespective of the synthesis route, NPs and NMs offer interesting prospects in attaching different functional groups on their surface with an aim to tailor their surface characteristics and subsequent actions. As discussed earlier, a key distinguishing feature of NPs and NMs over their bulk counterparts is their high surface area, which implies their high activity. Thus, it is critical that their surface activity is desirably controlled to modulate their interaction with external entities. To this end, a thorough understanding of their surface chemistry and on-demand modification of their surface properties have attracted considerable interest [28]. Overall, it is now possible to functionalize NPs and NMs with non-covalent or covalent interactions, including electrostatic, van der Waals, hydrogen bonds, hydrophobic interaction and π - π stacking [46]. The covalent modification of NPs and NMs typically involves modification with molecules containing specific functional groups, such as carbonyls, amines and thiols to, for instance, control their surface

charge. These surface charges can then influence the ability of NMs to selectively interact with oppositely charged entities and play a role in different biological applications [47]. Similarly, non-covalent interactions may also be employed to achieve similar objectives. Such functionalized NPs with a well-defined chemical surface may find different applications in many important fields and for different purposes [40]. For example, in the biomedical field, they may find applications in targeted chemotherapy and drug delivery. These surface functionalization strategies have facilitated the encapsulation of drugs either inside the nanocarriers or the attachment of drugs to the nanomaterial surface [48]. For a more comprehensive understanding of how the physicochemical properties of NPs and NMs may influence their interaction with the biological world, the readers are directed to follow reviews in this area [28].

Nanosensors and nanobiosensors

Nanosensors can be defined as nanoscale devices that capture information from the macroscopic world and convey that information through the use of nanoparticles to assist with the detection of target molecules of interest [49]. In the case of nanosensors, the nano component acts as a transducer to assist with signal production. The analytes detected by a nanosensor may include physical analytes (e.g., ultraviolet radiation), chemical analytes (e.g., pesticides and other pollutants), and biological analytes (e.g., allergens, disease biomarkers and pathogenic organisms). A biosensor on the other hand typically employs a biological component, such as biomolecules or cells as a transducer to produce a signal with the assistance of other sensor components. However, it is also not uncommon in the literature for a biosensor to be defined as a device that is focused on detecting a biological analyte. Aligned with the above definitions of nanosensors and biosensors, a nanobiosensor can then be considered a device that contains both the nano- and bio-components. In certain nanobiosensors, the nano component acts as the transducer to produce a signal while the bio component acts as a molecular recognition element to identify the target. An example of this category of nanobiosensors includes nanoparticles that produce specific colours with the assistance of DNA aptamers as molecular recognition elements for specific target analytes [50]. However, in another category of nanobiosensors, in a nanodevice, a biochemical transducer produces a digital electronic signal proportional to the concentration of a specific analyte or group of analytes [49, 51]. The oldest biosensor was based on catalytic systems that integrate enzymes, cellular organelles, or entire cells. The signal was transduced by electrochemical, optical or thermometric principles [51]. The more modern biosensors are based on

DNA and RNA, antibodies, ligands, enzymes, aptamers, etc. [52]. The transduction mechanisms become more sensitive with the addition of piezoelectric and magnetic transducers [51]. A crucial point is that the sensitivity of the nanobiosensor is largely influenced by the nature of the biosensor's interface [53]. To increase the sensor's analytical capacity, the developments in nanobiotechnology have facilitated the incorporation of nanomaterials at the biosensor's interface [54]. The advantage of this kind of sensor resides in nanomaterial properties, resulting in compact and user-friendly devices with desirable properties of high speed, selectivity, sensitivity, and stability, along with lower production and operational costs [49, 55]. Applications of nanosensors and nanobiosensors cover many fields. In this section, we will focus on the last two fields, providing some examples of applications in environment and agriculture.

Applications of enzymes in nanobiosensor for the agricultural industry have been reviewed by Verma [57]. In certain cases, the enzymes were immobilized on the sensor by covalent or non-covalent bonds and directly interacted with target molecules. As an example, a nanobiosensor was developed by immobilizing acetylcholinesterase enzyme on a carbon nanotube to detect organophosphates at levels as low as 0.5 nM [56]. Such nanotechnologies have been deployed to detect various agrochemicals, wherein the use of nanomaterials has significantly improved the minimum detection limit down to the picogram range [18, 57–59]. Nanobiosensor can be applied not only for pollutants, but also find interest in pathogen detection. DNA-based biosensors, combined with an electrochemical transducer, can provide rapid detection with high sensitivity and selectivity for a given pathogen [60]. The principle of these nanobiosensors involves the formation of a hybrid sandwich between the detector's single-strand DNA labelled with electrically active magnetic nanoparticles, and the pathogen's single-strand target DNA. Through the redox properties of the electrically active magnetic nanoparticles, the target DNA is finally detected [60, 61]. Notably, the exploitation of electrochemical transducers represents only one of the available strategies for signal amplification, and other approaches such as colourimetric nanozyme sensors and nanoparticle-assisted microscopy-based approaches have also been proposed for pathogen detection [62, 63]. As previously reviewed [64], optical biosensors also exist, that can measure interactions between the target analyte and ligand through light. This kind of sensor may allow rapid detection of pathogenic agents in 10–15 min with a small number of samples. The most common strategy applied in optical detection is colourimetric approaches, fluorescence-based assays and surface plasmon resonance [64]. Optical detection can also be applied in

environmental monitoring of heavy metals through carbon quantum dots [65] or metal nanoparticles [41]. The optical characteristic of carbon quantum dots is deeply different from the properties of bulk carbon: carbon dots could be tuned easily and they show a remarkable fluorescence response in the presence of metal pollutants. In addition, their water solubility makes them suitable to detect water contaminants in industrial waste [52]. Similarly, when metal nanoparticles are employed for the detection of heavy metals, such as mercury, the change in the optical properties of these nanoparticles due to change in their morphology after interacting with heavy metals can be employed for rapid detection of mercury [41].

Nanoparticles in soil remediation

Using specifically engineered nanomaterials (ENMs), an innovative remediation strategy for polluted areas was conceived. The difficulties in the remediation of a polluted area lie in the heterogeneity and complexity of the soil–water interface, as well as in the efficiency of the interaction between the remediation agent and the target pollutant. The small size and highly reactive surface confer NPs the potential reactivity and versatility to fulfil this complex task. ENMs could remove effectively multiple pollutants through the combination of adsorption and redox degradation in a single step or with sequential treatments [66]. The strategy used in remedial applications takes advantage of the immobilization/adsorption process, for example in metal-contaminated soil [67]. The challenge in adopting this strategy is the selection of specific materials or coating compounds for the contaminant materials in relation to target and soil characteristics. Different NPs have been effective in the immobilization of organic and inorganic pollutants, such as carbon NMs, metal oxides, nanocomposite NPs and others; each NP interacts with a different target pollutant. Carbon NMs absorb organic pollutants by using π – π interactions and van der Waals forces, while metal oxide NMs (i.e. Fe_3O_4 and TiO_2 are the two most studied) and nanocomposite NPs (i.e. polysaccharide stabilized Fe–Mn binary oxide) immobilize heavy metals and organic compounds with surface complexation [66].

Another remediation strategy consists of photocatalytic degradation, where, under UV irradiation or sunlight the nano-photocatalysts help degrade organic contaminants like pesticides, polycyclic aromatic hydrocarbons or polychlorinated biphenyls in wastewater [67]. In practice, contaminants were extracted from soils with non-polar solutions and the collected leached solution was treated with photocatalytic NPs. The photocatalysts efficiency is highly dependent on various factors like irradiation

period, the pH of the solution, the concentration of the pesticides and the photocatalysts [68].

Titanium dioxide (TiO_2) is currently the most studied photocatalyst for mineralization of organic pollutants [68]. The potential application of this metal oxide as a photocatalyst in wastewater is supported by several positive evidence. The TiO_2 photocatalytic process at ambient temperature is often able to reduce organic pesticides into non-toxic compounds like CO_2 and H_2O . In addition, TiO_2 photocatalyst is cheap and can be functionalized with a variety of substrates, which favours its use in more than one situation. Magnetite (Fe_3O_4) is another metal oxide with a relevant importance in soil remediation, owing to its possibility to participate in Fenton reactions. To oxidize organic compounds, Fenton reagents were applied in the presence of ferrous ions, acting as catalysts against organic contaminants in wastewater [69]. Another application of the Fenton reactions with magnetite is the in situ remediation of contaminated soil through magnetite injections [70]. This approach allowed soil remediation without pH adjustment, representing an interesting alternative to the normal method where more than one reagent is applied; in addition, magnetite is a non-toxic mineral. Magnetite is structurally and catalytically stable, and this characteristic allows several oxidation cycles [70]. To increase magnetite activity, injection in the soil is followed by sonication to improve dispersion in the soil matrix and improve overall degradation efficacy [69]. Another interesting approach for the remediation of heavy metal-contaminated soils is represented by nanoscale zero-valent iron (nZVI) [66, 71]. Thanks to its strong reduction ability, nZVI forms complexes with heavy metals. For example, nZVI can make complexes with Cr(VI) that is reduced to Cr(III) thus forming, in most circumstances, precipitates like ferrous chromite [72]. The combination of nZVI and silicon-rich biochar could increase the removal activity of nZVI and improve the dispersion of the particles [73]. Similarly, the Fenton application of nZVI has also been exploited to degrade concentrated dye solutions that can contaminate soils and agricultural sites through industrial effluents [74].

Another purpose in using NPs in the soil is to improve its characteristics and performance. Currently, the study of the interaction between NPs and soil for its final stabilization is still in the early stage. However, studies on the addition of chemicals NPs to the soil showed improved soil characteristics by altering their physical or structural properties [75]. The soil particles interact with each other depending on the mineralogical and chemical compositions, and structural organization under the influence of the environment, e.g., soil organic matter and water contents: the relations between particles control soil physical behaviour. The addition of NPs can modify

the association between soil particles with an increase in soil plasticity, compaction, shear strength, consolidation, miscellaneous properties and a decrease in hydraulic conductivity. The use of nano-copper, alumina, and nano-clay showed a positive influence on soil parameters.

Nanofertilizers

Efficient fertilization practices in agriculture have become of crucial importance to sustain crop production for the ever-increasing global demand for food, and to combat the impacts of climate change. It is estimated that 40–70% of N and 80–90% of P of commonly used fertilizers are lost into the environment or are chemically bound to the soil, and are therefore unused and unavailable for plants [76]. This waste represents an enormous economic and environmental cost.

The properties of NPs can be exploited to improve plant nutrition strategies with a novel approach. The high surface-to-volume ratio of nanofertilizers allows for a simultaneous decrease in the number of fertilizers and to increase in their assimilation by plants. This brings a reduction of nutrient-related toxicity and fertilizer loss [77]. The synthesis of fertilizers at nano-metric dimensions is an opportunity to significantly reduce the environmental impact of traditional fertilizers. The approach of NFs synthesis involves the use of nanoparticles that contain nutrients and/or reduction to the nanoscale of the fertilizer granules.

NFs may be produced by top-down or bottom-up approaches, which can be further classified as chemical, physical and biological synthesis methods. Bottom-up methods mostly involve chemical synthesis, while top-down involve both physical and biological synthesis [78]. The process of biological synthesis takes advantage of the plant and microbial biomass leading to a more environmentally friendly procedure compared to the other two techniques [79, 80]. Moreover, NPs produced by biosynthetic processes are more cost-effective and are better taken up by plants [77, 78]. In this regard, some macronutrients are under the spotlight [e.g., nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulphur (S) and calcium (Ca)] and micronutrients that are needed by plants in trace/low amounts, but are crucial to maintaining correct metabolic processes [81]. Besides macro- and micro-nutrient NFs, some nanobiofertilizers can be defined as the intersection of a biofertilizer with a nanostructure or nanoparticles [81].

Due to the high potential activity of NFs, these must be applied at a correct concentration, because application at lower or higher concentrations may cause undesired physiological effects. For example, unwanted enzyme activities can be induced that could be either hazardous or toxic for the plant. Also, seedling growth can be

affected by improper NFs application, probably due to oxidative stress, visualized by toxicity symptoms [78].

It has been proposed that NFs can penetrate plant cells on the condition that the NPs are smaller than 8 nm; they can pass through cell wall pores, having a size between 3 and 8 nm. It is believed that aggregates larger than 8 nm cannot enter the plant cell [82]. However, this size range needs substantial validation, as extensive research in mammalian systems has demonstrated that even larger NPs can enter cells through various uptake mechanisms [83–85]. In plants, the access point in the leaves are stomata, hydathodes or cuticles [86]. Few data are available about NPs entry through hydathodes, but some studies demonstrated CeO₂ NP (8 nm size) accumulation inside lettuce leaves [87] and the negatively charged polymer poly(ϵ -caprolactone) NP entering into *Brassica juncea* through these openings [88]. The knowledge of NP access through stomata is wider and supported by different studies. Stomatal uptake is dependent on morphology, physiological status and plant–environment interaction [86]. Cuticle of the leaves can also potentially contribute to taking up NPs; even if the cuticle represents a barrier and decreases NPs mobility and uptake into the mesophyll. It was hypothesized that 4–100 nm TiO₂, Ag, Pb and polymer NPs can cross the wax layer [86]. However, in other cases, Fe₂O₃, Pb, or CuO NPs application on leaves could generate necrotic spots caused by the release of metal ions.

Regarding the interaction of NPs with roots, particle size, shape and charge play a pivotal role in the uptake process [89]. NPs encounter plant root surfaces, characterized by a rough texture containing mucilage, exudates and small chemicals; hence, NPs have to interact with typically negatively charged exudates. Also, the cell wall itself has a negative charge, therefore NPs with positively charged surfaces are likely to be more prone to be absorbed and accumulated on the root surface [90, 91]. Uptake of TiO₂ NPs with diameters smaller than 140 nm was observed in wheat roots and 36 nm NPs were translocated to leaves [92]. The epidermal cell wall of the roots presents small pores that may prevent absorption of large-size NPs, but NPs with a diameter between 3 and 5 nm can penetrate plant roots by passive absorption driven by capillary force, osmotic pressure or direct passage via epidermic cells [93]. This scenario includes the aggregation and transformation of NPs by root exudates that impact root uptake and bioavailability. This mechanism is dependent on the chemical properties of NPs, exudates and rhizosphere microenvironment [90]. Finally, different plant species and plants at different developmental stages secrete different exudates that affect NPs absorption; this factor generates an even more tangled scenario [90]. Overall, only a piece of limited

scientific information is available regarding the interaction and uptake mechanisms of NPs and NFs in different parts of a plant, and much can be learnt from an extensive amount of research already taken in the field of nanomedicine which suggests that size, shape, and surface properties of NPs can significantly influence their cellular interactions, uptake and toxicity in biological systems through various mechanisms [28].

Different organic and inorganic NFs were evaluated in the last years. Some of them showed side effects, other than the fertilizer purpose. As an example, Ti NF has manifested improvements in the properties of the soil after application, in turn, this soil amelioration had a positive influence on plant growth and biomass production [77]. Another example can be illustrated with Cu and Ag NPs. *Medicago sativa* showed better agronomical performances, including increased fresh weight and length of shoots and roots, in plants treated with Cu NPs in soil [94], whereas Ag NPs improved growth under heat stress and increased plant tolerance in *Triticum aestivum* [95]. These two metal species also show antimicrobial and pesticide properties: a study with *Solanum lycopersicum* treated with leaf application of Cu NPs showed an effective control of *Phytophthora infestans* [96], while Ag NPs showed antibacterial activity against plants pathogens such as *Xanthomonas campestris* and *Xanthomonas axonopodis* [97].

Nanopesticides

Conventional pesticides are usually diluted with water and sprayed. This operation exposes the active ingredient of the pesticide formulation to degradation by environmental factors that can significantly decrease its performance [24]. Furthermore, only a small amount of the applied pesticides reacts with pests, as the remaining is lost in the environment, with detrimental consequences on the environment and humans. This pesticide abuse could lead to emerging pesticide resistance of target pathogens, weeds and insects [1]. One of the goals of nanotechnology is to reduce the use of pesticides and pesticide-induced toxicity for the environment by reducing the active ingredients to a nano-dimension or by designing nanocarriers that induce slower release and increase stability [24, 98].

In a study conducted on *Uromyces viciae-fabae* treated with CuSO_4 and $\text{Na}_2\text{B}_4\text{O}_7$ NPs, it was shown how micronutrients can be used in plant disease management [99]. Metal nanoparticles such as Ag, Cu, ZnO_2 and TiO_2 manifest positive actions against bacteria, fungi and viruses [100]. Ag NPs, thanks to “green synthesis” have been intensively researched for their antifungal action which has been confirmed for different pathogens [101]. Application of Ag NPs has shown inhibition activity

against *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia lunata*. It is supposed that Ag NPs may kill fungal spores destructing their integrity, while inside the cell may interact with phosphorus and sulphur cellular compounds, causing DNA and protein damage [101]. Antibacterial effects related to CuO NPs have been ascribed to an overproduction of reactive oxygen species [102]. A further micronutrient with excellent antimicrobial properties is ZnO NP which exhibits effects against *Xanthomonas oryzae*, *Rhizoctonia solani*, *Podosphaera pannosa*, *Phytophthora capsici* [103]. Moreover, antiviral activity has been reported following the application of ZnO NPs in *Nicotiana benthamiana* by direct deactivation of tobacco mosaic virus (TMV) [104]. In vitro studies with TMV have shown significant aggregation and damage to the virus after the application of ZnO or SiO_2 NPs. When these NPs were applied daily by foliar spraying for 12 days on tobacco leaves, there was an activation of plant defence that inhibited TMV replication [104]. In plants, the metal NPs were observed to interfere at different levels with virus replication, and this activity was observed in both foliar and soil applications [105]. This direct action of metal NPs against pathogens represents an enormous potential for the agronomic sector, however, this property needs further studies.

Another class of nanopesticides is represented by pesticides bound to nanocarriers. Through the physical adhesion of the active molecule to a substrate, regulation of its activity can be obtained. The design of nanocarriers foresees that pesticides can be dissolved, adsorbed, trapped or encapsulated inside soft NPs (for example nano-matrices, polymer and lipids) or hard NPs (for example silica, nanotubes or graphene) [1]. A nanocarrier confers high stability to the pesticide by protecting it from photolysis and the degradative action of the environment. An interesting example is represented by novel nanopesticides containing avermectins (AVMs) as the active ingredient. AVMs are a group of natural drugs (non-toxic for mammals) produced by *Streptomyces avermitilis*, that can induce a larger flow of chloride ions into the cells, thus inducing hyperpolarization culminating in paralysis of the invertebrate neuromuscular system. The AVMs find a broad application as insecticides, nematocides, anthelmintics and arachnicides [106, 107], but are damaged by high-energy ultraviolet radiation [24, 54]. AVMs loaded in functional boron nitrile NPs exhibit several positive traits, including high loading capacity, efficient water-dispersibility, gradual/sustained release, pH-responsive release, superior shielding against UV degradation, and superior ability to adhere to plant surfaces [108].

Also, zein-based orbicular nanocarriers loaded with AVMs exhibit light protection, an increase in water

dispersion and wettability and adhesion of the pesticide [109]. Studies conducted on nanocapsules designed against nematodes and composed of ionic liquid of phosphonium, AVM and gel microcapsules demonstrate a controlled release of AVM as a function of pH and temperature, and greater stability in the soil [110]. AVMs represent an example, but the same strategy could be applied with other active ingredients. The nanoemulsion of ginger essential oil was shown to reduce the damage of *Xanthomonas oryzae* pv. *oryzae* under glasshouse trial. The essential oil caused abnormal growth and reduction in biofilm production. The nanoemulsion approach was applied due to the instability and poor solubility of ginger essential oil [111]. Nanotechnology can provide useful support to improve stability and permit the controlled release of pesticides against target organisms. In fact, several nanoencapsulation strategies have played a pivotal role in advancing nanomedicine by facilitating the delivery of hydrophobic drugs in an aqueous biological environment, minimizing drug degradation, and controlling the release parameters during drug delivery applications [112, 113]. Many of these principles can be adopted from the field of nanomedicine to that of nano-agriculture to advance this field.

A new frontier for the management of pests and pathogens is represented by RNAi. RNAi is a conserved eukaryote mechanism involved in different metabolic processes and in defence against viruses and transposons, which is also possible to redirect against different targets such as insects, bacteria, fungi and weeds [114–116]. Application of dsRNA loaded in layered double hydroxide (LDH) clay nanosheets has been tested against cucumber mosaic virus and pepper mild mottle virus [117]. Through degradation of LDH carrier, dsRNA is released into plant cells thus silencing the homologous RNA after topical application. One single spray application of this nanosystem has been observed to protect the plants for at least 20 days.

Risks of nanotechnologies in agriculture

Nanotechnology has many advantages that deserve to be exploited to solve agricultural problems; however, its indiscriminate application can lead to several problems and side effects [98]. The interaction of NPs with plants' metabolism and soil environment is a crucial point to be considered for this emerging technology. In the plant system, NPs may enter through leaves or roots. As reported in the review by Khan and co-authors, different application methods have been considered to investigate NP–plant interaction [27]. The seed treatment has been widely used to study these interactions, while soil and foliar application have also been used to examine the influence on plant growth. After entering vegetative organs, NPs interact at cellular and subcellular levels

with suppressive or stimulating effects, which are directly dependent on NPs characteristics (chemical nature, size, reactivity, and specificity) and quantities present in the plant. Negative effects of NPs and NMs include disturbance in the cell cycle, nucleotide damage, early growth of seedlings, growth inhibition, and activation of stress-induced signalling pathway [118, 119]. Metal NPs and carbon nanotubes NMs are known to exhibit phytotoxicity effects like inhibition of germination, changes in root and shoot biomass growth, ROS generation leading to oxidative stress, influence the morphology of the plant tissues, genotoxicity and cytotoxicity [120]. Therefore, all the applications of NPs in soil or plants could potentially represent a double-edged sword: optimal doses of NPs application need attention while a huge research gap exists in the area of NPs' physiological and environmental side effects.

The mitigation of the above-mentioned risks in the agricultural sector could be addressed into two successive phases. At first, a more detailed study on the interaction between NPs, plants, soil and microorganisms is needed to clarify the interaction of NPs with the environment. Literature reports promising recent studies in this sense [121, 122]. Secondly, it is crucial to study the correct inclusion of these new materials in the agronomic practice. This second step shall be based on the previous studies, but this field research represents the unavoidable step to avoid possible pollution or environmental damage. A strong interdisciplinary approach including nanotechnology, biotechnology and agronomy shall be applied to successfully bring this technology in the field.

Biostimulants

In 1997, Zhang and Schmidt defined biostimulants as materials that, in minute quantities promote plant growth [123]. This definition marks a clear border between these products and conventional fertilizer and soil amendments, which promote plant growth only in larger quantities. In that context, biofertilizers can also be considered distinct from biostimulants, as biofertilizers often contain a variety of living microbes that can enhance plant nutrient uptake by colonizing the rhizosphere and facilitating easier access of nutrients to the plant root hairs. With their proven profitability, environmental friendliness and composition, biofertilizers are considered as a viable alternative to the hazardous synthetic fertilizers [124]. However, a potentially synergic application of biostimulants and biofertilizer cannot be excluded, as reported in the co-inoculation of rhizobia microorganisms and biostimulants treatment in beans [125].

Nevertheless, the capacity of biostimulants to enhance plant characteristics with 'minute quantities' is mainly

due to the complex interactions with plants' physiological processes induced by these substances. The definition of "Biostimulant" has been updated in Regulation 2019/1009 of the European Parliament (EU, 2019) that lays down harmonization rules for various categories of fertilizing products. A biostimulant is defined as "a product stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency; (b) tolerance of abiotic stress; (c) quality traits; (d) availability of confined nutrients in soil or rhizosphere" [126]. A classification of biostimulants based on functional components is difficult to make, as they are obtained from various raw materials, generating a complex mixture where it is unfeasible to define specific bioactive compounds [127]. In one case, the classification of biostimulants products has been proposed based on seven categories: humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, beneficial bacteria, beneficial fungi, chitosan and other biopolymers, inorganic compounds [128]. At present, this still represents one of the most adopted schemes in biostimulant-related literature.

The biostimulants market in 2016 was valued at around EUR 1.45 billion, with a projected rise to about EUR 2.66 billion by 2022, with a compound annual growth rate of approximately 10.5%. There are two major categories currently dominating the global market, seaweed extracts and humic substances that together cover 64% of the total but the CAGR include all the category exposed above [129]. The quantification of the total area treated by biostimulants in 2016 was 14.3 billion hectares which were expected to rise to 27.6 billion hectares in 2022 [129]. Europe plays a key role covering 2016 40% of the total market.

An important source of biostimulants are organic waste streams, placing these products in the spotlight for innovation in agriculture. The concept of recycling organic waste from different industries to transform it into fertilizer products with a new economic value is core for the circular economy, aiming to decrease the material input cost and environmental impact of the agricultural sector. Building a new circular economy has also a central role in the European Green Deal. Agricultural and food industries produce a tremendous amount of organic waste, however, the best source candidates to become biostimulants are represented by waste that cannot be reused in pharmaceutical or chemical industries, where this biomass would be more valuable [130]. This reduces the current market disposability of biomass for waste-derived biostimulants [131]. In other instances, some biomasses cannot be recycled for pharmaceutical

or chemical products due to legal requirements. For example, tanning industries produce 450–600 kg of solid waste from animal skins to obtain 200 kg of leather [131, 132]. This animal skin waste can be submitted to hydrolysis to generate a mixture of free amino acids and peptides that can be used as biostimulants [127]. Diverse types of organic waste from a wide range of sectors can undergo similar hydrolysis and extraction processes, leading to various products with biostimulant properties. The characteristics of these hydrolyzed compounds depend on the process used, the degree of hydrolysis and the raw material [133]. This recycling possibility represents a small part of the greater opportunity available. The same circular strategy has been proposed for vegetable wastes generating plant extract biostimulants or in the fish industry to recover important molecules like chitin and chitosan [131, 134]. A further implementation of the recycling process consists of integrating two extracting methods when raw material might be the source of different valuable compounds. An example is represented by the use of seaweed as source material: through sequential extractions and lysis, products with different purposes can be simultaneously obtained: pharmaceutical and nutraceutical commodities, and biostimulants and biofertilizers [135].

Despite established evidence of biostimulant effects and economic importance, the physiological mechanisms behind their influence on plant physiology and molecular pathways are still to be fully delineated, depending on their complex composition, but also on the dose used, the application strategy and timing [12]. This overall complexity is currently studied by utilizing the synergic use of different omics-based analyses [136, 137]. This review will focus on the three most studied and economically valuable products, namely humic substances seaweed extracts and protein hydrolysates [138].

Humic substances

Humic substances (HS) consist of degraded organic materials, produced as byproducts of microbial metabolism, heterogeneous in their molecule combination, and found in soil and sediments [12]. HS due to their natural creation process are composed of highly diversified molecules and do not assemble in a regular sequence. The elements that compose HS have been largely studied and the range of elements that compose standard fulvic humic acids, according to the International Humic Substances Society (IHSS) are C from 50 to 60%, N from 0.7 to 5.1%, H from 3.5 to 4.8% and O from 31.6 to 45.5% [139]. However, the molecular compositions of HS are widely varied and the average of elemental composition changes, depending also on what kind of microbial degradation is involved and on soil composition [140]. Normally, HS

functional groups that play a pivotal role are the carboxylic and aromatic groups, the last ones giving stability against chemical and biological degradations [140]. Aromatic groups present in HS phenolic compounds have drawn the attention of several studies, due to their characteristics and properties. The content of phenolics, in particular polyphenolic compounds, is linearly correlated with major electron-donating capacities [141]. The distribution and abundance of redox functional groups active in HS are typically dependent on microbial decomposition and transformation of HS in soils [142]. With pH decrease, functional groups are protonated and repulsion increases; these events lead to molecule rearrangement in colloid structures with a disruption of weak non-covalent interactions [143].

Root exudates contain low molecular weight organic acids that aid the solubilization of HS along with their assimilation by root (Fig. 3) [144]. Carboxylic groups also contribute to HS solubilization and their biological responsiveness [145]. The solubilization of HS creates two different molecule types, namely high molecular size (HMS, >3500 Da) and low molecular size (LMS, <3500 Da), both interacting with the root. In particular, the HMS fraction is reported to interact with cell

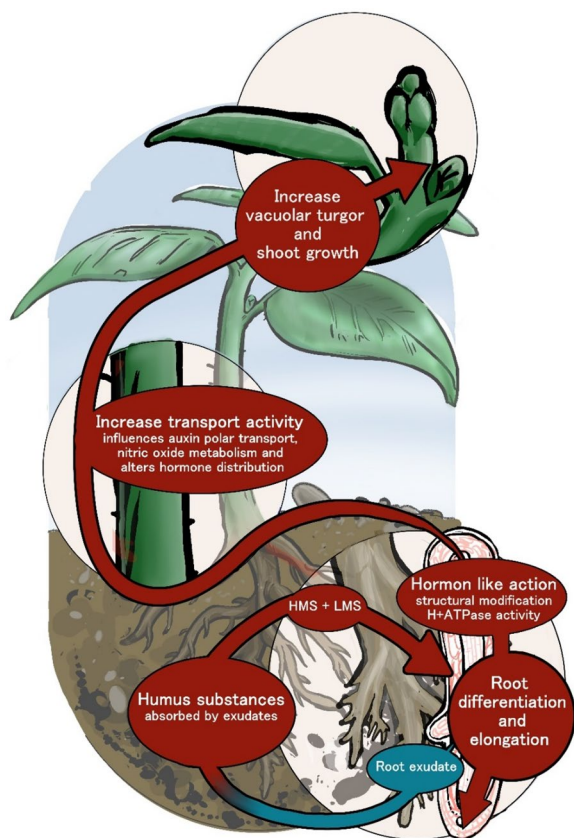


Fig. 3 Main physiological effects of humic substances biostimulant activity and putative absorption mechanism

wall components and root membrane receptors to activate an internal signal transduction cascade, while LMS is more easily absorbed because of their small dimension [146]. The principal target of HS is the root, the organ where the plant response is most effective, where elongation and increase in root biomass are often reported. This observed stimulatory effect is likely due to a structural modification in the central cylinder that favourably impacts water conductivity and nutrient flux rate, whereas, in the cortex, the increased resource storage boosts diameter [140]. The hotspot of HS action in root formation is represented by the elongation and differentiation zone of the root which includes small, densely meristematic cells that are in continuous metabolic activity and are more susceptible to lateral root formation [147]. Besides inducing structural modifications, HS influences auxin polar transport, and nitric oxide metabolism and alters hormone distribution in plants [148]. In other studies, HS structure was found to contain indole-3-acetic acid (IAA) and different molecules with IAA-like activity [149]. Interaction of IAA-like molecules with root cells involves increased activity of root plasma membrane (PM) H^+ -ATPase, vacuolar H^+ -ATPase, H^+ -pyro-phosphatase (H^+ -PPase) and cross-induction of NO generation that accumulates in pericycle cells [143]. Activation of this pump contributes to preserving H^+ electrochemical gradient to provide osmotic pressure to the vacuole to adequately enable water uptake and turgor maintenance. Activation of root PM H^+ -ATPase also appears crucial to increase lateral root development [147]. Roots of *Zea mays* treated with humic acids (HA) show a clear stimulation of the vanadate-sensitive ATPase activity just like the formation of an ATP-dependent proton gradient [147]. The induction of PM-bound ATPase activity results in an accumulation of H^+ in the apoplast; the resulting acidification leads to the activation of pH-dependent cell wall loosening enzymes, thus promoting cell enlargement; active H^+ secretion is compensated by the absorption of K^+ ions to ensure turgor pressure during cell elongation [150]. The effects of HS treatment are not limited to roots, but also extend to the aerial part of the plant. Root treated with purified HA induced a substantial increase in shoot growth that was correlated with an enhancement in root H^+ -ATPase activity, an increase in nitrate shoot concentration paralleled by a decrease in roots. These effects were associated with marked increases in the shoot concentration of cytokinins and polyamines, with a consequent decrease in roots [151].

Seaweed

Seaweeds are marine, photosynthetic algae that are abundant in every ocean. There are three main seaweed phyla: Phaeophyceae (brown algae), Rhodophyta (red

algae), and Chlorophyta (green algae), each phylum composed of thousands of species [126]. Seaweeds have been used traditionally as food, folk remedies, dyes, and fertilizers. The exploitation of seaweeds has taken place for several centuries and in diverse cultures. In the last 50 years, there has been a global increase in the demand for macroalgae for different purposes, mainly for biomass production or extraction of products used in pharmaceutical, biotechnological and agricultural fields [135]. Hydrocolloids, such as alginate, carrageenan and agar, are still the most commonly exploited components for their gelling properties in foods, pharmaceutical and biotechnological applications [152]. In terms of biological activity on plants, the main known elicitors from seaweeds are cell wall polysaccharides: in green seaweeds consisting of ulvans, agarans and carrageenans; while in brown seaweeds comprising alginates and fucans as well as the storage polysaccharide laminarin [153–155]. Some of these polysaccharides (e.g., fucoidan, carrageenan, and ulvan) are modified by different sulphate groups, thus producing diversified structures, also leading to a variety of specific properties [156]. Cell wall polysaccharides in seaweeds also contain a wide range of organic and inorganic molecules that are known to contribute to their biostimulant activity.

The extraction phase largely remains the most decisive step to guarantee the integrity of the biologically active molecules with biostimulants effects [157]. A large number of extraction procedures for the production of either liquid or soluble powder forms have been developed by various commercial entities, most of which are proprietary [158]. The identification of more effective extraction methods should consider the complexity of the seaweed composition and assure the biological activity of biostimulant molecules. The two main existing strategies to obtain biostimulants from seaweed rely on physical methods (heat, pressure, and microwaves) coupled or not with chemical methods (solvents, acids, and alkali). The most widespread extraction process is based on alkaline treatment at high pressure. The advantage of this strategy consists of optimal and effective extraction of polysaccharides with moderate degradation in oligomers, however, some hormonal molecules can degrade [126]. To avoid this problem, various innovative extraction technologies have been tested: ultrasound-assisted extraction, enzyme-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, and pressurized liquid extraction [158, 159].

Seaweed extracts contain several bioactive ingredients; nevertheless, their positive effect cannot be exclusively ascribed to any of them no in particular [126]. Recent research demonstrated that the full extract operates in synergy to trigger a comprehensive positive reaction

in the plant, whereas each component acts on different metabolic networks either independently or interactively. In general, seaweed extracts induce a cascade of reactions inside the plant, leading to overall growth and improved resistance against biotic and abiotic stress [126]. For example, treatment with *Ascophyllum nodosum* in different plants shows how this extract interacts with different plant metabolic pathways. A microarray study conducted on rapeseed treated with *A. nodosum* extract showed the differential expression of genes involved in nutrient transport, cell metabolism and stress response [160]. In that study, a physiological analysis of nitrate metabolism showed enhancement of NR enzymatic activity and an increase in the plant dry weight. Although no clear effect on photosynthesis was found in that study, treatment with *A. nodosum* appeared to enhance the dark phase of photosynthesis, with the genes involved in carbon fixation, such as Rubisco or carbonic anhydrase, being mostly upregulated. This increased carbon fixation was also demonstrated by a larger number of starch granules in treated plants, as observed by TEM.

A study conducted on tomato and bell pepper showed the effects of different seaweed extracts on major hormonal biosynthesis genes. Seaweed extracts were able to enhance plant growth with a significant concomitant increase in the expression of genes implicated in auxin (IAA), gibberellin (Ga2Ox), and cytokinin (IPT) biosynthesis [126]. Plant responses to seaweed extracts might be related to the presence of plant hormones in the biostimulants and/or its ability to trigger the production of hormones in the plant, as reported for the HS and the protein hydrolysates [126].

Besides its effects on plant growth and development, research and industry have a major interest in abiotic and biotic stress responses in treated plants. Zhang et al. reported an increase in superoxide dismutase activity after seaweed extract treatment in bentgrass [123]. It was hypothesized that such a phenomenon could be attributed to the hormonal and osmolyte effects of the extract. Similar responses were shown by foliar application of *Ascophyllum nodosum* extract on a *Paspalum vaginatum* cultivar, during drought stress and under saline conditions [161]. Seaweed extract-treated plants showed increasing lipid peroxidation and significantly higher SOD, catalase and ascorbate peroxidase activities ascribed to a defence strategy to reduce ROS. This abiotic stress tolerance was ascribed to a series of cumulative and synergic effects: plants showed better photochemical activity attributed to the mineral composition of seaweed extract and the presence of growth regulators like cytokinin and abscisic acid; enhanced root extension, modulation of root architecture leading to increased nutrients uptake and transport; increased carbon fixation, the

greater buildup of nonstructural carbohydrates and thus improved energy storage; metabolism and osmotic adjustment and enhanced proline accumulation [126, 157].

Besides abiotic stress responses, seaweed extracts were reported to recruit in plants several defence mechanisms against biotic stressors. The cell wall polysaccharides of seaweeds like ulvans, laminarins, and carrageenans and their derived oligosaccharides have been shown to induce some resistance responses to pathogens in plants [126]. Active polysaccharides present in seaweed extracts can elicit specific responses including upregulation of various PR proteins, defence genes and enzymes belonging to different pathways leading to the development of induced resistance. Compositional differences in seaweed extracts related to the origin species could be the real reason for the differential defence pathway induction.

Protein hydrolysates

Protein hydrolysates (PH) as biostimulants are an innovative technology with promising applications as confirmed by several studies conducted on a wide range of horticultural and agronomic crops. PHs are 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis' [162]. PHs are mostly produced by chemical (acid and alkaline hydrolysis), thermal and enzymatic hydrolysis from a broad spectrum of animal and plant biomass [128]. Chemical hydrolysis of proteins by acid or alkaline treatments at high temperatures (121–137 °C), is a severe process resulting in a high content of free amino acids and the hydrolyzation of several amino acids (like Lys, Trp, Ser, Thr, Cys), and conversion from L-forms to D-form of amino acids, limiting their metabolic effects and sometimes causing other toxic effects in plant metabolism [163]. As an alternative with lower energy requirements, PHs are produced by enzymatic proteolysis, ensuring better control of the final product. Specific proteases optimized for peptide/oligopeptides proportion, yield, and other physicochemical characteristics are employed as almost all PH characteristics, including biostimulant properties, are strongly influenced by the type of protease hydrolyzing the starting material [133]. Another major source of chemical variation in PHs is represented by the protein source e.g., collagen from leather byproducts, fish byproducts, legume seeds, and alfa-alfa biomass. For example, collagen-derived PHs composition is dominated by amino acids like glycine and proline while aspartic and glutamic acids are dominant in legume-derived and fish-derived PHs [164]. These compounds are marketed as liquid extracts, soluble powder,

or granular form, and are applied as foliar sprays or as a lateral application near the root.

Commercial and experimental PHs have been tested on different vegetable products such as ornamentals, fruit trees, horticultural vegetables, and arable crops. Many of these protein-based biostimulants have positively enhanced plant growth and yield, but have also improved marketability traits, such as product quality and nutraceutical characteristics [133]. These effects have been attributed to the activation of plant enzymes related to N and C metabolisms, to support high growth rates and biomass productivity. Another study showed the effects on nitrate metabolism of foliar spray application of a legume-derived PH under optimal and sub-optimal nitrogen concentrations in tomatoes [165]. In foliar spray-treated plants, the transcript levels of the ammonium and amino acid transporters and nitrate reductase (NR), nitrite reductase (NiR), and ferredoxin-dependent glutamate synthase (GLT) were significantly upregulated. An upregulation was also observed for glutamine synthetase 1 (GS1), glutamine synthetase 2 (GS2) and glutamate dehydrogenase (GDH) transcripts in the leaf after application. Furthermore, other studies also showed an increase in secondary metabolites such as phenols in pepper and anthocyanins in apple [133]. The whole mechanism of action of PH biostimulants in plants remains unclear, however, biologically active peptides have been isolated and chemically characterized from PHs, especially those derived from plant materials. Evidence suggests that specific amino acids and/or peptides in hydrolysates could mimic the action of naturally occurring peptide hormones in plants also behaving like signal peptides. For instance, a short peptide (12 amino acids) called 'root hair promoting peptide' has been identified in a legume-derived PH [164]. A metabolomic analysis of tomato cuttings treated with the lightest fraction of a legume-derived PH has evidenced an increase in secondary metabolites (phenylpropanoids, terpenes, flavonoids, nitrogen-containing compounds, glucosinolates and alkaloids) with a similar metabolic signature of indole-3-butyric acid (IBA) treated plants, suggesting an auxin-like activity [166]. A complex alteration of the plant's transcriptome and proteome was confirmed by another study on collagen-derived PHs with two different concentrations applied to maize roots [136]. These authors observed an alteration in the expression of 1006 genes, as well as 242 differentially abundant proteins. Most of these genes and proteins were linked to plant stress tolerance and important processes for both plant growth and development like metabolic pathways, ROS-related systems, phytohormones, transport and cytoskeletal reorganization.

Other biostimulants molecules

The scientific attention on biostimulants has also favoured the discovery of new molecules of different origins with biostimulating capabilities, in addition to humic substances, algae and hydrolysates. The list of these molecules is ever-increasing and has a great variability. In the present, review we focus on molecules that are easily available, cheap, and have a good potential to be easily integrated with the nanosystems. These can have an organic origin, like polymers such as chitosan and lignin, or can be an inorganic compound such as silica.

Several works reported the biostimulant capacity of chitosan on plants. Chitosan is a natural and cheap biopolymer produced from chitin, the major constituent of arthropod exoskeleton and fungi cell walls and the second renewable carbon source after lignocellulosic biomass [167]. Chitosan contributes to the control of the presence of pathogens and the induction of plant defence reactions. The high biological activity of chitosan in the induction of plant immune response may be related to the processes taking place in plant cell walls [168]. Chitosan can induce the synthesis of H_2O_2 acting in plant cells as a signal molecule in defence reactions to stress and, in turn, increase the activity of superoxide dismutase (SOD), peroxidases (POX), polyphenol oxidase (PPO) and catalase (CAT), enzymes involved in the direct scavenging of ROS [28]. This effect of chitosan on the plant depends on the species, developmental stage, and physiological condition. Furthermore, the molecular weight appears to be the most important parameter with a great impact on the level of biological activity of chitosan [168].

Another interesting organic polymer is represented by lignin. A study showed how the pretreatment of the seed with lignosulfonates had a positive effect on soybeans grown under heat stress. Their work reported that under controlled conditions, the germination rate of soybean seeds incubated at 35 °C increased after treatment with the lignin derivative [169]. The lignin stimulant was represented by phenol-rich materials, which can stimulate metabolic processes leading to better seed development. This effect can be traced back to the hormonal-like action that the phenolic compounds, contained in the lignin, can exert on the biochemical activities of the early-stage shoot [170, 171].

Not only organic materials can stimulate the metabolism of plants, but silica NPs have also been observed to behave as a biostimulant. Silicon is considered a non-essential element for plant nutrition, however, silicon can be applied as a biostimulant in horticulture via foliar spraying, incorporation into the soil or fertigation [172]. Silica NPs improved plants' stress tolerance in different case studies, such as salt stress, drought, heavy metal,

pest and diseases [173]. The silica particles have also been shown to increase plant resistance during osmotic stress [174], but their function within the plant's metabolism is not entirely clear. These initial results on the role of silica particles as biostimulants suggest that many other inorganic NPs and NMs may also play a role as a biostimulant, either in isolation or synergistically in association with organic biostimulants. This may open exciting opportunities for implementing nanotechnology alongside biostimulant-facilitated improved agricultural production.

Overall, biostimulant observed effects on plant growth and stress tolerance are the tangible results of a wider, more complex and elaborated metabolic engagement. More information on the physiological and molecular effects of these substances begins to emerge, and the scientific community is gaining a deeper understanding of the effects and the potential application of biostimulants in agriculture.

However, more research is needed, as plant responses vary between different plant species, and knowledge about biostimulants that activate specific pathways in plant metabolism is still fuzzy. Urgent research on biostimulants could follow two parallel lines: on the one hand, there is a need for more field research, conducted in real environments, as farmers need to have information to make their decisions; on the other hand, more data on the specific components within biostimulants that elicit plant responses together with results on the physiological mechanism could provide suggestions for designing even more efficient substances.

Nanoencapsulation

A further improvement of the NP technology is the optimization of substance delivery mechanisms using encapsulation. The need to improve the efficiency of the control of overactive molecules has attracted the attention of scientists for a long time; already in the 1980s, in the medical field, the application of starch microparticles as carriers were proposed, to prevent degradation during injections and improve the delivery of low molecular-weight molecules or proteins to target organs [175]. Two different types of albumin-loaded starch microcapsules were tested, demonstrating that the loss of the loaded molecules follows the starch degradation of the microcapsule, which underlines the importance of the ability to control the release of a specific molecule on a target [175].

Thanks to the successful application of various nano-platforms in medical care, a broader range of nanoencapsulation strategies has been designed and applied over the years such as nanoprecipitation, emulsification process, polymerization and layer-by-layer nanoparticles

[176]. The difference between medical and agricultural applications lies in the nature of the environment in which these platforms interact and in the active ingredients used. However, the goal of increasing stability and release control is unchanged for both sectors. Prudent use of NMs is essential to exploit the beneficial aspects

of NMs in agriculture while minimizing eco-toxicological effects [120].

Wang et al. classified nanocarriers into four groups based on the loading principle: molecular level loading, surface loading, matrix loading, and cavity loading systems (Fig. 4) [177]. Molecular level loading systems

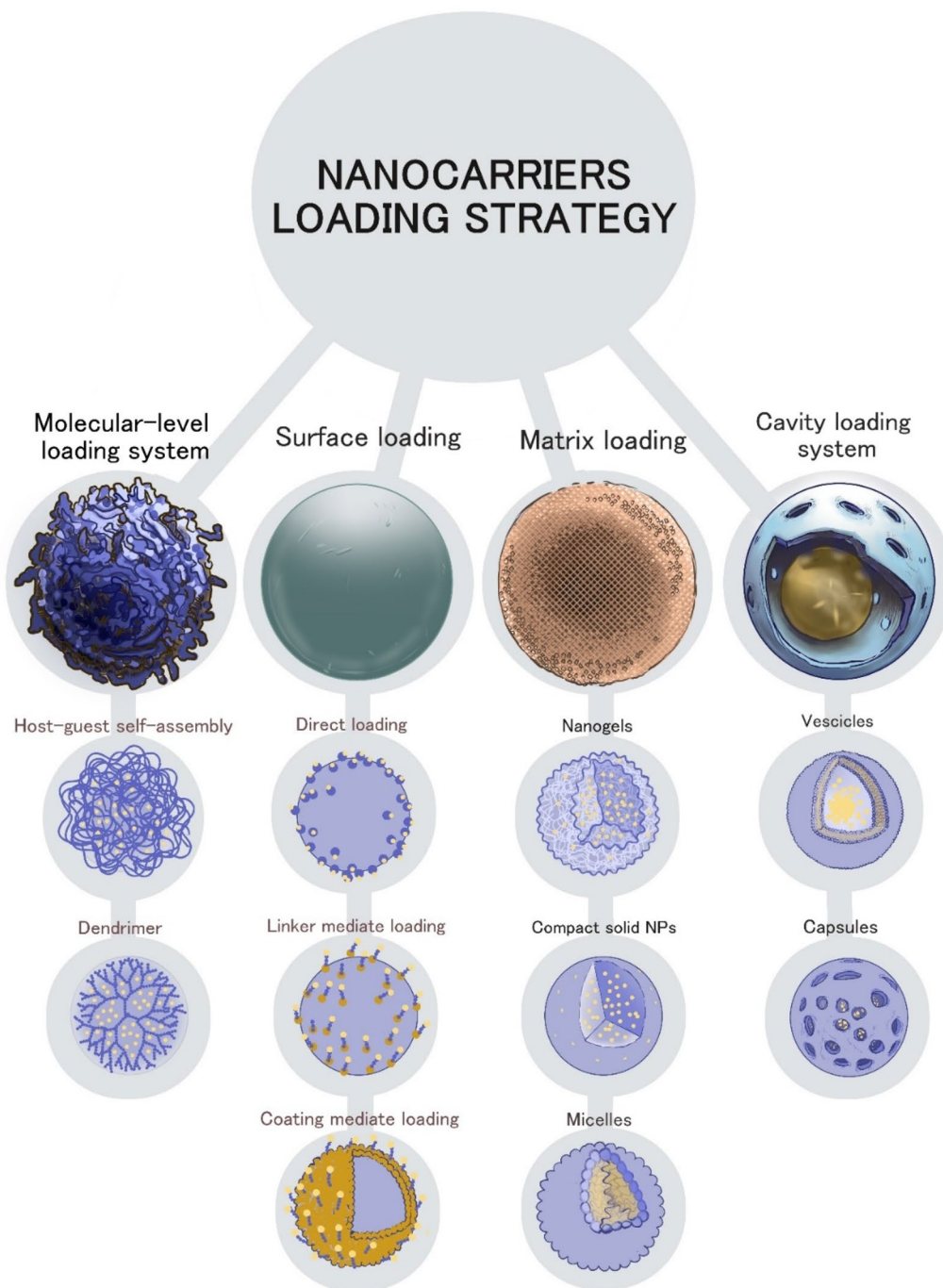


Fig. 4 Schematic illustration of the different nanoencapsulation strategies

are a popular method consisting of the retention molecules and host molecules. Retention molecules are composed of small molecules, polymers, or peptides, while guest molecules can be low molecular weight molecules with an active ingredient such as drugs or nucleic acids. The interaction can be physical or chemical in nature between these two parties, which could produce complexes. The complex created by the physical interaction is self-assembling due to the hydrophobic interaction forces that drive the process. The derived structures are often cyclic molecules having a hydrophobic internal cavity and hydrophilic edge or there may also be a dendrimeric complex. The loading capacity depends on the polymer characteristic that defines the pore capacity, and on the functional groups present in the polymer structure. Target molecules can be charged both by hydrophilic interaction with a hydrophobic pore and/or by weak chemical interactions with the functional group of polymers. Host molecules loaded with chemicals are often used to design intelligent release systems, while physically loaded nano-components, due to the non-specific nature of the interaction, can hardly achieve controlled release. In terms of loading, only small molecules can be loaded with this technique; furthermore, the content is usually not high due to the size limits of the host body cavity. These limitations are even more pronounced in the case of loading by physical interactions: only certain sizes and specific guest molecules can be loaded, so content loading is more limited [177].

An example is the application of *Toddalia asiatica* essential oil based on nanoencapsulated chitosan which exhibits antifungal activity and inhibits aflatoxin B1 [178]. The NPs of chitosan-based nanoencapsulated oil were prepared using the ionic gelation method, in which *Toddalia* essential oil was sequentially added dropwise to the low molecular weight chitosan solution [178]. Negatively charged sodium polyphosphate (TPP) ions, that electrostatically interact with positively charged chitosan (NH_3^+ groups) were used to generate highly reactive chitosan-TPP ions, which react with *Toddalia* essential oil thus forming chitosan-based nanoencapsulated oil NPs. SEM images showed that the chitosan NPs were compact, soft and spherical and the chitosan possesses significant encapsulation efficiency and load capacity with essential oils [178]. Furthermore, the strong electrostatic interaction between the chitosan NPs and the negative charge of the fungal cell wall allowed for easy penetration. The chitosan polymer protected essential oils from degradation and guided them inside the cells.

Solid particles consist of inorganic materials, for example, Ag NP, graphene, carbon sheet, and quantum dots. These objects possess a high surface/volume ratio inversely proportional to their diameter; an intact

surface and physicochemical properties of the NP surface (charge properties, polarity and chemical reactivity) influence the loading and functionalization of the vector. Loading of the target molecule occurs through different strategies. The simplest strategy involves the direct loading of a molecule onto the surface of the NPs; in this case, the molecule can be loaded with non-covalent interaction or chemical bonds. The indirect loading of the target molecule can be done using physical interactions, hydrophobic interactions, or chemical bonding. To release the chemically grafted target molecule, it is important to control the kinetics of the reactions to break the covalent bond and allow for a controlled release, which is better achieved using intermediate molecules between NPs and the target molecules; this is termed as linker or coating mediated loading strategy. But the presence of many functional groups on the surface could affect the surface charge and change the colloidal stability of the NPs. Moreover, through the formation and breaking of chemical bonds, negative effects on the steric hindrance of the target molecules on the surface and inside the cavities may result. This chemical interaction between NPs and target molecules could eventually lead to unwanted chemical modifications of both [177].

Hao and colleagues reported a functionalization strategy for 2-D Boron Nitride nanoplatelets loaded with the pesticide AVMs [108]. To obtain these NPs, they relied on the simultaneous conjugation of 2-D BN nanoplatelets (BNNP) with 3-mercaptopropyl trimethoxysilane (MPTMS) and on the condensation reaction with polyethylene glycol.¹⁰⁵ Through the combination of conjugation and condensation reactions under hydrothermal conditions, composite BNNP: PEG/MPTMS nanocarriers were obtained, separated at the nanoscale level. The AVMs could be effectively loaded thanks to hydrophobic-hydrophobic interactions and π - π stacking interactions with nanoplateform to BNNP: PEG/MPTMS. In this case, the release was controlled by pH. In the presence of an acidic environment, there was a reduction in the release of AMNs, whereas an alkaline environment promoted the deprotonation of functional groups of the pesticide and the hydroxyl groups of the carrier, which led to strong electrostatic repulsion with highly increased release of AMNs.

Matrix loading is the term that refers to delivery systems where the release of the incorporated molecule is dependent on the gradual dissolution of the material that composes the matrix structure. Most of the target molecule is hidden within the matrix, while only a small part is exposed on the surface. The critical factor that defines release activity is represented by material decomposition or degradation rate, which depends on the reaction with the surrounding environment, and the surface/volume

ratio of the matrix. The host molecules present on the surface can leave the matrix by diffusion, the speed of which depends on the diffusion distance and the size of the pores. To facilitate diffusion, smaller molecules with shorter diffusion lengths and large exposed surfaces are used. Host molecules not only influence loading and release characteristics, but also influence biochemical behaviour in vivo. Loading of the matrix system can generally be achieved by two strategies: the pre-loading method, in which the target molecule is co-precipitated/assembled with the excipient or precursor to form the matrix; the post-loading method, when the matrix structure is prepared in advance, and is followed by incorporation of target molecules. A matrix classification can be done according to the structural characteristics of the matrix; it can be divided into nanogels, compact solid nanoparticles, and micelles [177].

A nanocomposite biopesticide was produced via nanoemulsification and freeze-drying process, using whey protein as a nanocarrier matrix to encapsulate azadirachtin, a natural insect-killing compound extracted from neem seeds [179]. The prolonged/permanent localization of some azadirachtin in the internal compartments of whey β -lactoglobulin could be attributed to chemisorption between a reactive thiol group of Cys121 and several functional groups of azadirachtin to form organosulfide derivatives. The combination of slow-release and non-release functions could provide short- and long-term protection for plants. Indeed, the release behaviour followed exponential kinetics and took 2 days to reach the plateau.

The last method of nanoencapsulation is the cavity loading system which refers to encapsulation in a hollow vector with an internal void, where the shell protects and prevents the release of the target molecules. This strategy allows for the provision of a larger cargo space than the matrix strategy. The release of the contents occurs through the rupture of the shell or the opening of the pores on the surface of the shell. The basic structures that define these systems are vesicles and capsules. The vesicles are characterized by hydrophilic hollow vectors in double-layered shells, while the capsule has a single-layered shell. The shell has a different degree of porosity which is influenced by the composition of the material and the method of preparation of the shell [48]. On the surface of the shell, the pores can be small and do not allow the release of molecules, or they are large enough and some of these pores can dominate the diffusion process [177]. For example, there are hollow porous silica nanospheres (HSNs), which have been unveiled as potential vectors for pesticide delivery. However, these HSNs characterized by tiny mesopores on the shells suffer mainly from the post-synthesis pesticide loading process

[180]. To eliminate this disadvantage, Nuruzzaman et al. hypothesized that the development of a through hole or pore opening on the shells could bring about an effective loading of active molecules on the HSNs using a simple dipping method. HSNs with a single large through hole or pore opening on the shells were later called bowl-structured hollow porous silica nanospheres. This bowl structure was designed to load imidacloprid pesticide. The release time could be identified as the delay time required for diffusion from the inner core into the medium, driven by osmotic pressure followed by dissolution and pesticide release. This nano-platform allowed to increase the pesticide action time. Another field of application of nanoencapsulation, besides fertilizers and pesticides, is represented by biostimulants. In general, controlling the level of product delivery and increasing the activity time can further decrease the material input and the cost of agricultural production [181].

Nano-biostimulants

Biostimulants can protect the plants against environmental factors and improve the productivity and profitability of the crop without impact on the ecosystems. But these products have limitations in large-scale applications, one of the key limitations being their easy degradability. Biostimulating substances are often diluted in water for foliar or root application. This step could expose the molecules to the degrading action of biotic and abiotic processes that could drive a decreasing efficacy and lower time of action in plant. An interesting strategy to overcome these problems is the use of nanoencapsulation techniques. The union of the potential of the biostimulant loaded in specifically engineered nanocarriers is still unexplored and only a few applications are present in this innovative field [182] (see Additional file 1: Table S1). The first study was conducted 20 years ago, in which a-naphthaleneacetate (NAA), a regulator of plant growth, was loaded by the self-assembly technique in an inorganic matrix of double hydroxide layers of Zn–Al. The release of NAA from the matrix occurred due to the pH variation in the solution [183]. That work aimed to explore the capacity of the release mechanisms; however, no tests were conducted on plants with this nanoplatform. It took some time to have an application of nanoencapsulated biostimulant molecules in plants. The use of chitosan NPs loaded with the NO donor *s*-nitroso mercaptosuccinic acid (*s*-nitroso-MSA) has been reported to alleviate the effects of salt stress in maize [184]. Treatments of salt-stressed maize plants with 50 μ M and 100 μ M *S*-nitroso-MSA NP chitosan on soil produced a higher foliar *S*-nitrosothiol content than treatment with the free NO precursor mercaptosuccinic acid. The low-concentration treatment of *S*-nitroso-MSA chitosan

NPs completely prevented the salt stress alteration of photosystem II activity, the chlorophyll content, and the growth of maize plants. Thanks to their 38 nm size and positive zeta potential these nanoplateforms were effectively taken up and translocated into the plants from the roots. The size of 38 nm allowed the possibility to enter through the root tissues and to be allocated to the leaves [92, 184]. The chitosan S-nitroso-MSA NP within the plant tissue underwent a spontaneous thermal decomposition that led to the diffusion of NO, resulting in the formation of a non-toxic dimer of mercaptosuccinic acid. This excessive presence of NO in plant tissues, caused by the presence of the nanosystem, significantly increased the activity of S-nitrosogluthathione reductase (GSNOR). The stimulation of GSNOR and other enzymes correlated with NO/NO-S homeostasis, and this activity was also induced by various abiotic stresses [184]. The same NP carrier of chitosan, but loaded with SA, was assessed in a different work, where salicylic acid-chitosan nanoparticles (SA-CS NPs) were obtained with ionotropic gelation, the same technique used previously by Oliveira et al. These SA-CS NPs exhibited 35.2% and 68.1% release of SA in 12 and 96 h, respectively, at pH 4.5 [185]. The controlled release based on the protonation of chitosan allowed the effect of SA on plants to be extended, and the association in nanodimensions of chitosan and SA increased its positive action with lesser use of hormones than the use of the two single compounds alone. Application in maize of SA-CS NPs contributed to cellular redox homeostasis, and NPs also stimulated the activity of plant defence enzymes such as peroxidase (POD), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) which have key roles in lignin biosynthesis. The treatment also promoted leaf area, shoot–root length, root number, stem diameter and chlorophyll content in treated plants. In field conditions, treated plants showed improved disease control and a significant increase in plant height, cob length, grain yield/plot and 100-grain weight. These results were attributed to the slow release of SA from NP SA-CS, which could prevent sudden exposure of plant cells to SA, thus also preventing any toxic effects while stimulating plant growth. Furthermore, the chitosan component of NPs also exerts its bioactivity through the stimulation of plant growth and disease protection. Another work with alginate/chitosan nanocarriers loaded with the growth factor gibberellic acid (GA) provided encouraging results in field tests [186]. Another group reported the synthesis of nanocarriers composed of the plant hormone indoleacetic acid (IAA) by intercalation into layered double hydroxide (LDH) via the co-precipitation pathway [187]. This strategy protected the active molecule from enzymatic degradation and the biological activity of IAA was preserved. The synthesis of

1-triacontanol self-assembly on LDH nanocarriers, with improved dispersion and sustainable growth regulating properties in the foliar application, was also reported [188].

All these works have reported the use of growth factors as host molecules loaded in different vectors and highlighted how nanotechnology can enhance the action of the biostimulants, opening new scenarios and possibilities. The possible synergic effect between the transported host molecule and the vector is because chitosan is a natural molecule that induces numerous biological responses in plants, including the defence responses to abiotic and biotic stresses, depending on its structure and concentration [167]. Observations in many plant species treated with chitosan indicate the induction of plant defence enzymes and the synthesis of secondary metabolites, such as polyphenols, lignin, flavonoids and phytoalexins. In addition to protecting SA and allowing its controlled release, chitosan NPs could also support and amplify the stimulatory metabolic effects of SA.

In recent years, the application of nano-products combined with biostimulating molecules has evolved from a pioneering and exploratory phase and has begun to be more concrete: the capping strategy is a technology that already exists and is successfully used in various sectors. As reported in the recent review by Jiménez-Arias, the encapsulation strategy, already found in the agricultural sector, could be applied to biostimulants with various natural polymers and various encapsulation techniques [189]. A further opportunity to enhance the delivery and controlled release of biostimulants consists in nanoencapsulating the active molecules with eco-friendly substrates, for example, cellulose, fly ash, clay-based, iron oxide and hydroxyapatite nanoparticles of varied sizes. These nanocarriers can come from waste materials that represent an unrecognized and untapped resource. In this regard, nanocellulose holds a huge potential, since it possesses high water absorption and retention ability, as well as excellent mechanical properties; nanocellulose can be conveniently extracted from forest and agricultural residues, algae waste, or industrial byproducts [190]. Another example could be represented by fly ashes from power plants: thanks to their high silica content derived from coal, they can be used to obtain mesoporous silica materials widely used in nanoencapsulation [191]. Moreover, silica NPs could promote plant growth and development by increasing photosynthesis and plant nutrient absorption rate in adverse environments and could increase mechanical barriers, thus creating in plants a first line of defence against environmental stressors. In plants, silica NPs could induce the production of a range of defensive compounds, and the expression of defence genes [173]. The example of fly ashes highlights one of

the several possibilities of combining the recycling of waste material with the possible biostimulating effect of the carrier. Another potential source of porous silica from natural waste materials could be the husk of various cereals, such as rice husk which is used as a fuel in various agri-processes. The members of the Poaceae (grass) family naturally accumulate a substantial amount of silica during plant growth as a part of their defence mechanism. Previous research has shown that such natural forms of silica can be harvested in the form of nano-silica particles through biological synthesis and bioleaching processes [42, 43]. Another potentially recyclable material to combine with biostimulants for nanoencapsulation is lignin. Lignin has already been used for the nanoencapsulation of AVMs and a recent study has revealed its biostimulating activity [171, 192]. Further studies are needed in this direction, as there are still many unclear aspects of the interaction of biostimulants and nanosystems with plants and the environment. From a theoretical point of view, all the experiments reported in this paragraph could have advantages and disadvantages, as many aspects need to be considered in developing these products, such as final plant efficiency to enhance production, loading capacity, eco-friendly of the NPs productions, and also the cost of production. Based on the data reported in the papers it is difficult to encompass all possible advantages and disadvantages. However, some pivotal points to drive the future direction of this research field as the above-mentioned aspects, can be fixed. Finally, the intrinsic potential of integrating biostimulants with nanotechnologies could represent an invitation to a new concrete response to the growing problems of food production while respecting the environment.

Future perspectives

The application of nano-biostimulants in the agricultural sector could lead to a revolution in the cultivation approach and in the way of treating waste materials in agriculture and beyond, in line with a green economy dimension. Nanomaterials with embedded biostimulant molecules increase the possibility of identifying new biostimulant sources from any raw material, allowing field application of volatile or easily degradable molecule, which, without the support of NPs, could remain relegated to laboratory experimentation. Additionally nano-biostimulants could represent a revolution for agronomic crop treatments: a single product could induce double effects on the plants: one response due to the encapsulated biostimulant; a second reaction from the capsule itself.

Further research in this area should be based on an interdisciplinary expertise, including plant physiologists, experts in nanomaterial development and agronomists.

The design of nano-biostimulants derived from this interdisciplinary approach should focus on a deeper understanding of the behaviour of NPs and biostimulants in the plant while pushing towards environmentally friendly and cheap biostimulants and nano-capping materials, possibly from waste substances. Promising compounds, in this regard are, for example, lignin and silica extracted from crops waste. Coupled with basic research, applied studies for field responses are crucial to allow for the farmers' adoption of this new promising technology.

The nano-biostimulants could contribute to a new way of thinking the agriculture, based on circular economy, decreased human environmental impact, more integrated with the producing territory.

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Supplementary Information

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Additional file 1. Published biostimulant-based nanosystems.

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Author contributions

PM: conceptualization, writing—original draft, visualization; AM: writing—original draft; supervision; RS: writing—review and editing; VB: writing—review and editing; supervision; project administration; funding acquisition; PC: conceptualization; writing—review and editing; supervision. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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