Safe nanotechnologies for increasing effectiveness of environmentally friendly natural agrochemicals

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Abstract

Natural compounds and living organisms still have a limited use in crop protection, and only a few of them have reached the market, despite their attractiveness and the efforts made in research. Very often those products have negative characteristics compared to the synthetic compounds, e.g. higher costs of production, lower effectiveness, lack of persistence, and inability to reach and penetrate the target plant. Conversely, nanotechnologies are having an enormous impact in all human activities, including agriculture, even if some nanomaterials are not environmentally friendly to produce, or could have adverse effects in the agriculture and the environment. Thus, some nanomaterials could facilitate the development of formulated natural pesticides, making them more effective and more environmentally friendly. Nanoformulations can improve efficacy, reduce effective doses, and increase shelf-life and persistence. Such controlled release products can improve delivery to the target pest. This review considers some available nanomaterials and nanotechnologies to be used in agriculture, discussing their properties and feasibility in the perspective of their use in sustainable crop protection, in particular to improve the effectiveness of natural bio-based agrochemicals.

1. Introduction

Biological constraints (e.g. fungal and bacteria pathogens, viruses, arthropods, and weeds) are responsible for major losses in quality and yield of crops and grasslands. Effective pest management represents a major challenge in modern agriculture, with a need to consider control efficacy, cost affordability, environmental safety, toxicity towards non-target organisms, and sustainability of the production system. Despite the progress in many technological fields, most of the practices for managing these constraints are still based on the use of synthetic chemicals. However, a large number of pesticides have already been withdrawn for regulatory reasons because of their hazardous effects in the ecosystem or in the food chain, or because they have become ineffective, due to increasing pesticide resistance.[1] These compounds are not being effectively replaced, causing serious difficulties for farmers in managing pests. As a consequence, there is a renewed interest in the development of alternatives to synthetic pesticides.

2. Potential and limits of natural agrochemicals - two faces of the same coin

Organisms interact with each other, protecting themselves from the others' attacks or combating the others' defence barriers, by producing an enormous number of mostly still unexplored secondary metabolites; e.g., allelopathic compounds, phytoalexins, antibiotics, repellents, fungal toxins, antifeedants, and insecticides. These chemicals are the result of co-evolution of the producing organism and its biotic environment, and could represent an extraordinary source of new biologically active compounds, with novel chemical structures and mechanisms of action, to be used in crop protection. Isolating and identifying these compounds has been an arduous task in the past, but modern instrumentation (e.g. high throughput screening systems or advanced analytical equipment) and sophisticated approaches (e.g. “omics” tools) have simplified this process and reduced its costs.[2] Even though many natural compounds have been described, many have yet to be discovered. There are some good examples of natural products used as herbicides (e.g. bialophos produced by *Streptomyces higroscopic*), insecticides (e.g. spinosyns, a family of macrocyclic lactones derived from species of the actinomycete bacterium *Saccharopolyspora spinosa*), and fungicides (e.g. strobilurins named from *Strobilurus tenacellus*, a wood-rotting fungus from which the first compound in this group was isolated), and many reviews are available on this subject.[3,4]

Despite the potential of natural metabolites to be used as safe and environmentally friendly agrochemicals, some of their characteristics often concurrently represent possible constraints and limiting factors for their practical application (Table 1).[5] For example, natural agrochemicals could offer novel chemical structures with new modes of action, but they these compounds are often too complex to be obtained by an affordable synthesis. They are obtained by from living organisms, but in many cases in very modest amounts, or the purification procedures are too expensive and/or not really environmentally friendly. They are believed to have minimal environmental impact, but this characteristic is usually associated with a short half-life due to instability or excessive biodegradability, making them commercially unattractive. Natural agrochemicals can be too specific or slow acting, or may not reach the *in vivo* target. Sometimes they have to be applied at very high rates, making them too expensive or hard to apply. Thus, although the list of promising or proposed natural agrochemicals is enormous, their market is still quite limited, being less competitive and satisfactory than synthetic agrochemicals.

3. Nanomaterials in agriculture

Nanotechnology could help change this scenario by developing new tools to improve effectiveness of natural bioproducts and by overcoming the weaknesses and the factors limiting their use (Table 2). Nanoscale-based delivery systems usually range in particle size from 1 to 100 nm, although in pharmaceutical science nanoparticles can be up to 1000 nm. Different definitions have been proposed for nanomaterials. In 2011 the European Commission defined a nanomaterial in a more-technical but wider-ranging way, as: “a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range of 1–100 nm” (https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32011H0696&from=EN). According to the International Organization for Standardization (ISO), a nano-object is “a discrete piece of material with one, two or three external dimensions on the nanoscale, i.e. ranging from approximately 1 to 100 nm.

Nanoparticles are nano-objects with all external dimensions on the nanoscale, where the lengths of the longest and shortest axes do not differ significantly. If the dimensions differ significantly, typically by more than a factor of three, other terms, such as ‘nanofibre’ (two external dimensions in the nanoscale) or ‘nanoplate’ (one external dimension on the nanoscale) may be preferred to the term nanoparticle (https://www.iso.org/standard/54440.html). However, an “agreed” standard definition of nanomaterial is still an open question, and will certainly have a strong influence on regulatory development and industrial/research interests (see above). In this regard, a few years ago, the European Food Safety Authority (EFSA) committed a study aimed to prepare an inventory of nanotechnology applications in the agricultural, feed and food sector.[6] As reported, nanomaterials can have an organic or inorganic nature, or be derived by a combination of the two. The main possible approaches by using nanomaterials in crop protection are: (a) the direct use of inorganic nanomaterials as nanopesticides (NPs), such as noble metals or silicon-based materials; (b) the use of natural/synthetic nanoscale delivery systems, such as natural polymers, to better deliver active ingredients (AIs); and (c) the formulation of the currently available agrochemicals at a nanoscale dimension, by preparing improved nanoformulations and nanodispersions.[7]

Although the use of nanomaterials can bring significant benefits to the agro-food sector, some health and safety issues must be considered. The risk of using these technologies is mainly related to the small size of the nanoparticles and their large surface area to volume ratio that increases their reactivity, which could: cause easy dispersion, cross anatomical barriers, reach more distal regions of the animal or human body, and display potential toxicity. In the agriculture sector, handling of nano-fertilizers and pesticides, which can be easily dispersed into the soil, water, or atmosphere, may increase the health risk of applicators and also increase environmental risks as well. Thus, designing low-toxic, biodegradable and eco-friendly nanoparticles would be necessary.

3.1. Solid nanoparticles as nanopesticides and pesticide carriers

Many different nanoparticles have intrinsic pesticide properties and thus have been considered both as potential active ingredients (nanopesticides) and as nanocarriers for the delivery of AIs.

3.1.1. “Inert nanomaterials”

This group includes a number of materials (amorphous nanosilica, nanoclays, nanohydroxyapatite) of natural or synthetic origin, that have been considered eco-friendly pesticides because they act mainly by physical mechanisms, being physio-sorbed by the cuticular lipids and disrupting the protective epidermis layer.[8] For example, different amorphous silica nanoparticles (SNPs) were proved to be more effective than bulk silica against the rice weevil *Sitophilus oryzae*.[9] Surface-charged, modified hydrophobic silica NPs were successfully used to control some agricultural insects and ectoparasites of veterinary importance.[10] They were successfully applied as a thin film on seeds to decrease fungal growth and boost cereal germination. Application of silica NPs on the leaf and stem surface did not alter either photosynthesis or respiration in several groups of horticultural and crop plants. They did not cause alteration of gene expression in insect trachea and were, thus, qualified for approval as nanobiopesticides,[11] although their toxicity remains to be understood.[12] A novel formulation based on silica NPs has also been proposed for improving the effectiveness and slowing the release of the pro-insecticide chlorfenapyr with promising results. Field tests showed that the insecticidal activity associated with silica NPs was at least twice that of chlorfenapyr associated with microparticles or without particles and the insecticide release was slowed down to over 20 weeks, providing high-localized concentration over a long time.[13]

Mesoporous silica nanoparticles (MSNs) are synthetic particles possessing well defined and tuneable pore sizes (2–50 nm), large pore volumes, high surface areas with easily-modifiable surface properties, chemical stability, resistance to microbial attack, tailorable nanostructures, biocompatibility, and aqueous degradability.[14,15] Moreover, MSN protect loaded active ingredients (AIs) against enzymatic degradation since no swelling or porosity changes occur in response to external stimuli, such as, pH and temperature.[16] MSNs are excellent pesticide delivery carriers, as their structural properties can be modified to either enhance or slow down release kinetics.[17,18] MSNs were studied for storage and controlled release of the fungicide metalaxyl. The fungicide, loaded into MSN pores from an aqueous solution by a rotary evaporation method was released in soil and water very slowly over 30 days.[19] In other studies, MSNs proved to be effective carriers for the delivery of the natural pesticide avermectin, being able to protect the AI against UV degradation and to slower its release, dependently on the pore diameter and shell thickness.[20]

Diatomaceous earth (DE) is almost pure amorphous silicon dioxide, made up of fossilised phytoplankton. By milling, a fine, talc-like powder or dust is obtained, extremely stable and not reactive, considered non-toxic to mammals. It acts as an insecticide by absorption of epicuticular lipids and fatty acids, leading to desiccation in arthropods.[21] Recent research has focused on enhanced DEs with other insecticides to allow control at low doses. For example, a mixture of DE with the plant (*Celastrus angulatus*) extract bitterbarkomycin (sesquiterpenes) was evaluated against the grain pest *Rhyzopertha dominica*, and found to be effective at dose rates as low as 150 mg/kg of wheat.[22]

Nanostructured alumina (NSA) was discovered and proposed as an effective insecticide against two grain pests, *Sitophilus oryzae* and *Rhyzopertha dominica*. It was more effective compared to a commercial diatomaceous earth product.[23] More recent research on NSA, synthesized using a modified glycine-nitrate combustion process, revealed that the mechanism of insecticide action is based on physical phenomena rather than on biochemical mechanisms. Moreover, particle size, surface area, and morphology are key factors, which determine insecticidal efficacy. Modifications of the synthesis route could allow achieving better results for the targeted species.[24,25]

Nanoclays are thin sheets of organic silicate material (in the order of 1 nm thick and 70–150 nm wide) produced from montmorillonite clays commonly found in volcanic ash. Their size is reduced and surface modified to form bio-compatible and low-toxic nanoclays. They have been successfully used as carriers for the plant growth regulator α-naphthalene acetate and for the controlled release of the herbicide 2, 4-dichlorophenoxyacetate.[26] They were further studied as a carrier for the natural antibiotic cinnamate,[27] which is a problematic agrochemical because of its rapid degradation in soil and the high dosages necessary for its effectiveness. When loaded in nanoclays, it proved to be released more slowly and to be retained in the soil for a longer period. This indicates excellent promise of nanoclays to be used for slow/targeted delivery of pesticides and fungicides, as well as DNA (see above).[28]

3.1.2. Metal nanoparticles

Different metallic and metal oxide nanoparticles - MNPs (copper, silver, titanium oxide) have been considered as antimicrobial agents. Because of their larger surface/volume ratio and their crystalline structure, they more effectively trigger biological responses compared to the traditional ionic form of the metals.[29-31] Although in some studies MNPs proved to be less toxic to mammalian cells than their corresponding ionic forms and to have a prolonged effect as a source of elements in an organism, as well as reduced risks for the environment and non-target organisms, their possible use in large amounts for agricultural purposes is still an open debate. Their production costs and regulatory obstacles are also issues that must be solved. Improvements in the process of MNPs synthesis have been obtained by including organisms in their production. This green synthesis has some advantages compared to other methods, as it is less costly, scalable for large production, and avoids waste of energy and the use of harmful toxic substances.[32] Hence, new strategies using bioactive materials from various biological sources are of special interest. Groups of different microorganisms including fungi, bacteria and yeasts, and plant extracts are being used to produce NPs.[33,34] By using this approach, recently *Trichoderma*-mediated Selenium nanoparticles (SeNPs) were synthesized and used for controlling downy mildew disease in pearl millet.[35] The antimicrobial activity of those MNPs seems to occur via: (1) photocatalysis-absorbed photons leading to the release of superoxide radicals, which cause the death of bacterial, fungal, and viral organisms by oxidation of critical molecular structures; (2) accumulation and dissipation in the cell membrane, leading to membrane damage and release of cell contents; and (3) uptake of metallic ions into cells followed by disruption of DNA replication.[36]

Silver nanoparticles (AgNPs) have been studied for a relatively long time. They are the most active MNPs, with both bactericidal and fungicidal efficacy. For instance, they were tested in lab conditions against *Raffelea* sp., the fungal causal agent of oak wilt [37] and against a number of other plant pathogens.[38] However, the potential side effects and the environmental impact of these MNPs remain to be determined. A number of microorganisms, e.g. plant growth promoting rhizobacteria (PGPR) were also used for the biosynthesis of AgNPs. For example, a strain of the bacterium *Serratia* sp. isolated from agricultural soil showed the potential to synthesize AgNPs with strong antifungal activity against *Bipolaris sorokiniana*, the spot blotch pathogen of wheat.[39]

The antibacterial potential of photocatalytic nanoscale titanium dioxide (TiO_2) by itself or augmented with other metals was evaluated against *Xanthomonas perforans*, the causal agent for bacterial spot disease of tomato. The absorption of photons by TiO_2 resulted in the creation of free electrons interacting with water molecules to create highly chemically reactive hydroxyl and superoxide free radicals.[29] The extent of microbial killing varies as a function of the target organism, the intensity of illumination, the efficiency of photo-catalysis, and the duration of exposure. Both TiO_2 and Zn have been reported to have lower ecological and toxicological risks at the application rates investigated than copper-based bactericides in normal use. TiO_2 occurs naturally in soils and in highly purified form in many commercial products over decades, and is classified as nontoxic.

In other studies,[40] copper nanoparticles were encapsulated and stabilized with highly biocompatible gelatin that is expected to be advantageous for interaction of the particles with cell membranes and their subsequent entry into the cell cytosol. Those NPs performed better than the equivalent amount of their precursor $CuCl_2$ against Gram-positive and Gram-negative bacteria. More recently, the same authors[36] showed in *E. coli* that the effect of NPs was due to multiple toxic effects such as generation of reactive oxygen species, lipid peroxidation, protein oxidation and DNA degradation. Copper NPs also have *in vitro* synergistic effects against some fungal phytopathogens when combined with copper-based fungicides.[41] However, evaluation of environmental fate and possible adverse effects against non-target organisms under field conditions should be determined.

3.2. Nanoemulsion and nanodispersions

One of the main limitations with the use of synthetic or natural pesticides is that they are generally poorly water soluble, so they need to be dispersed in a liquid phase for application. This makes necessary the use of large amounts of organic solvents to dissolve them. One way by which the problem is solved in commercial formulations is to combine the pesticide with a surfactant, thus increasing solubility for a suitable efficacy and uniform application in the field. In the case of liquid pesticides, these formulations are called emulsifiable concentrates, while solid pesticides are referred to as wettable powders. Typically, particle sizes for these formulations are in the micron range (1–20 µm in diameter). However, this approach has some disadvantages such as increased costs, more environmental pollutants, and user risks. Nanotechnology offers some more environmentally friendly and sustainable alternatives such as dispersing the AI in a liquid as a colloid in the form of nano-sized droplets or solid particles, stabilized with the aid of surfactants.[42] Nanoemulsions may be of the oil-in-water (O/W) or water-in-oil (W/O) types, depending on whether the oil is dispersed as droplets in water, or vice versa.[43] Solid lipid nanoparticles (SLNs) are other promising carrier systems are that can be used to transport nonpolar substances whose mobility is restricted by interaction with the lipids.[44] Although the main objective is to improve water solubility, recent research has shown improvements of other important properties of the pesticide, e.g. (1) increase in bioavailability, due to a combination of greater surface area for exposure and enhanced penetration into the target;[45] (2) enhanced stability, e.g. by protection from UV lights [46] or from hydrolysis;[47] and (3) controlled release, to slow the release process, resulting in more sustained exposure and longer term efficacy.[48,49] Numerous reviews have compared AI nanoemulsions and nanodispersions to the respective traditional commercial micro-formulations,[42,50] and many others have considered their technological properties or fabrication methods. Many studies have also been carried out in order to improve effectiveness of natural agrochemicals by using these approaches.[51-53] Being an enormous field of research, only a few examples have been discussed.

3.3. Polymer-based nanopesticides

Polymer nanoparticles and nanocapsules are composed by natural or synthetic polymeric materials. Some of them have a desirable trait: they are biodegradable. The substances that can be used for the synthesis of these nanodevices include starch, polypeptides, albumin, sodium alginate, chitin, gelatin and cellulose amongst others (Figure 1). The first work in this area began about 50 years ago by a German group [54] looking for pharmacologic applications.

Chitosan is a polymer that can be obtained by treating chitin from shrimp and other crustaceans with a base (alkaline substance), producing a polymeric β-glucan. It is well known as an elicitor of defence responses in plants and possesses antifungal properties, which makes it very attractive for applications in plant protection.[55] However, it can be used as a carrier for pesticides when synthesized in the form of nanoparticles, either alone [56] or in combination with other polymers.[57] This double function as nanocarrier and active substance itself, in addition to its origin from a waste by-product from the fishing industry, turns chitosan into a promising material for nanoformulating natural compounds.[58]

Alginate is another linear β-linked polysaccharide isolated form the brown algae group Phaeophyceae (commonly known as seaweed). It can be treated in several ways in order to produce hydrogels, microspheres, nanoparticles and nanocapsules,[59] and combined with other polymers such as chitosan,[57] producing a highly versatile system for nanoformulation of agrochemicals. Experiments under field conditions with insecticides (imidacloprid) have shown that a reduction in the dose of the active ingredient can be achieved, whereas the effectiveness of the treatment is not compromised and is even improved in time.[60]

Following with carbohydrate polymers, cyclodextrins are cyclic oligosaccharides composed of between 6 and 8 glucose subunits (named α, β and γ cyclodextrins, respectively). They have a hydrophobic core and a hydrophilic shell, and self assemble in aqueous solutions with other components to form nanoparticles and aggregates. In addition, cyclodextrins can be conjugated with other nanomaterials, enhancing their characteristics as nanocarriers.[58,61] They have been successfully loaded with fungicides [62] and tested for treatment of fungal plant diseases in the field,[63] or combined with fungicidal natural compounds such as geraniol.[64]

Plants store energy in the form of starch, and being one of the most abundant biomass materials in nature, it has multiple applications in industry. Nanostructures from starch have been developed, leading to different results depending on the protocols, mostly producing nanocrystals and amorphous nanoparticles.[65] They have been used for delivering nucleic acids inside plant cells [66] or producing slow release of insecticides [67] and fertilizers through nanocomposites.[68] This role for protection and slow release of the active components makes starch-based nanodevices quite attractive for combining them with natural compounds.

Lignins are cross-linked polymers of phenolic compounds that are constituents of the plant cell walls. Nanoprecipitation methods with lignin produces nanoparticles that protect the coated materials against corrosive agents [69] and degradation by UV and oxidants.[70] They can increase the efficacy of herbicide application [71] and open interesting possibilities for protecting natural compounds against degradation by external agents. Additionally, many plant pathogens such as fungi produce specific enzymes for degrading cell wall constituents (e.g. lignins), so these nanocarriers could be targeted and degraded specifically at the places where the fungal pathogen is acting, releasing the active ingredients.

Viruses have long been postulated as a means for pest control,[72] but more recently they have emerged as polyvalent nanocapsules capable of auto-assembly and carrying different substances ranging from drugs to nucleic acids.[73] Specifically, plant viral particles are gaining importance in this role, and some formulations have been developed and tested for delivering pesticides[74] and natural compound[75] against parasitic nematodes.

There are many other biodegradable polymeric nanocarriers being developed that show promising results for their use in nanoformulations for pesticides or natural compounds, such as those based on zein,[76] cellulose,[77] lipid/protein nanodisks [78] or syntetic polymers (e.g. poly-ε-caprolactone).[79] Moreover, particularly intriguing for the practical “consequences” are the studies on nanopolymers that are triggered under specific environmental or biotic conditions.[80]

4. Toxicity and environmental impact

The idea of using nanomaterials for field applications in agriculture must be addressed carefully in order to avoid creating new problems while solving other problems. For that reason, determination of toxicity and potential negative environmental impacts of nanodevices is needed before approval. One of the best ways to avoid this obstacle is turning to nanomaterials that have been proven innocuous and safe for human consumption. However, this in not always a guarantee that massive application in the field of a product already used by the food industry will not have negative environmental effects, as it is the case of silver nanoparticles.[81,82] Toxicity is a relevant question to be tested before using a nanodevice for agricultural applications. Direct toxic effects of nanoparticles are usually associated with their chemical composition and high specific surface area (high reactivity), which makes them biologically reactive.[83] However, it is important to differentiate if a compound produces cytotoxicity or it is toxic for the whole organism (acute or chronic). Because of their high reactivity, some nanomaterials can be occasionally cytotoxic and lethal for individual cells, but their effect on the whole organism is negligible and innocuous.

Nanomaterials can have damaging effects on plants, on other organisms, or affect environmental processes. In the case of plants and algae, negative consequences can involve alterations in photosynthesis due to several factors, such as reduction in light availability and gas exchange leading to decreased CO_2 fixation,[84] or directly inactivating the plant photosystem and affecting the electron transport chain.[85] Additionally, plant growth and physiology can be negatively altered,[86] and DNA damage (genotoxicity) has been reported.[87] Either terrestrial [88,89] or aquatic fauna [90] can be severely affected when exposed to certain nanoparticles at high concentrations. Also soil microorganisms playing important beneficial roles, such as mycorrhizal fungi [91] and bacteria [81] can show negative responses to the presence of some nanomaterials in their surroundings. These effects can lead to altered properties in the soil such as microbial respiration, transport of liquid and/or gases, and failed symbiotic relationships. Finally, nanomaterials may directly influence environmental processes such as altering precipitation by acting as nuclei for raindrops,[92] interacting with pollutants and, consequently, altering their toxic effects,[93] disrupting nutrient cycles,[94] or detrimentally affecting water purification.[95]

An important consideration about nanotoxicology is the experimental design. It is not easy to develop sets of assays that provide reliable information about realistic conditions. Securing the right dosage, proper way of application, exposure time, and parameters affecting the performance of the nanomaterials in the different media (such as size, agglomeration, mobility, precipitation, etc.) are key factors that might compromise the validity of the results.[96]

In general terms, an ideal nanodevice for using in agricultural applications should comply with the following traits: firstly, being non-toxic and environmentally safe, in order to avoid further contamination problems and a negative perception from consumers; secondly, synthesis and production of nanodevices must be easily up-scaled; thirdly, they should be made with low cost materials, in order that farmers can afford the cost of the new nanoformulated products and that they are not more expensive than the current agrochemicals.[97]

In order to clarify the needs and conform the procedures, very recently EFSA has prepared a Guidance on risk assessment of nanoscience, nano-objects and nanotechnology applications in the food and feed chain on humans and animals. The document provides more insights to physicochemical properties, exposure assessment and hazard characterisation of nanomaterials, suggesting how to establish whether a material is a nanomaterial, the key parameters that should be measured and the methods and techniques that can be used for characterisation of nanomaterials.[98]

5. Nano- and bio-technological approaches for developing a new generation of agrochemicals

In the last two decades, RNA interference (RNAi)-based technology has resulted a powerful tool for engineering pest-resistant crops,[99] opening the door for new agrochemical design. The application of the RNAi technology is based on the delivery of double-stranded RNA (dsRNA) or small interfering RNA (siRNA) to gene silencing. Thus, RNAi can be considered as a natural gene-based technology for highly specific pest control. The use of RNAi in pest management has been widely studied in different organisms showing the potential utility of this technology in both basic and applied science. For instance, expression of transgenes in wheat plants for production of dsRNA targeting fungal genes coding for MAP-Kinase and cyclophilin caused a pronounced reduction of the leaf rust infection by *Puccinia triticina*.[100] Immunity to *Fusarium graminearum* was observed in transgenic barley targeting the sterol 14α-demethylase (CYP51) genes of the fungus.[101] In addition, when using this RNAi technology to silence a critical gene for survival of an insect pest, resistance of the transgenic plants was observed.[102-104] Indeed, a new genetically engineered corn based on RNAi technology was developed and already approved by the US-EPA. It will reach the market soon and will help the US farmers to control the corn rootworm (CRW).[105] Finally, this RNAi technology has also been used against viruses, bacteria and nematodes.[106-108] However, either because of political, regulatory, or technical difficulties, transgenic crops are not always a viable solution. Hence, topical application of dsRNA for pest control is emerging as an appealing alternative to genetically modified crops. One of the most large-scale field studies was conducted by Hunter *et al* [109] to test the ability of the topical delivery of a dsRNA product to protect honeybees from infection by the Israeli Acute Paralysis Virus, IAPV. The product was used as a food additive for over-wintering bees with outstanding results regarding mortality and overall health. Topical spray delivery of dsRNA *in planta* has been successfully reported to target insect pests feeding on the plant.[110,111] Soil applications for root absorption or trunk injections have also been addressed with positive results on gene silencing, confirming that plant root can take up dsRNA and trunk injections facilitate the delivery of dsRNA through xylem and phloem.[110-112]

The rapid degradation of naked dsRNA and it has been a major challenge towards its practical application. In general, dsRNA is much more stable than single-stranded RNA, but it must be rapidly taken up in the cells and digested into siRNA. Therefore, the use of nanomaterials as carriers to reduce dsRNA degradation and to increase the cellular uptake of intact dsRNA has gained relevance lately. Recently, Mitter *et al* [28] demonstrated that dsRNA can be loaded on non-toxic, degradable, layered double hydroxide (LDH) clay nanosheets, known as "BioClay". Once loaded on LDH, dsRNA does not wash off, shows sustainable release and can be detected on sprayed leaves 30 days after application. Moreover, this study confirms that dsRNA could be translocated to untreated parts of the plant affording virus protection even after a single spray. Another type of non-toxic and easily biodegradable nano-carrier is the polymer chitosan. Zhang *et at* [113] loaded RNAi in the mosquito *Anopheles gambiae* using chitosan/dsRNA nanoparticles through larval feeding. Additionally, a cationic core-shell fluorescent nanoparticle (FNP) has been successfully utilized as an efficient dsRNA carrier to knock down key developmental gene expression and kill insect pests.[114] Other types of materials used for dsRNA protection with positive results are, liposomes,[115,116] guanylated polimers,[117] carbon quantum dot, and silica nanoparticles.[118]

The use of nanomaterials for improved delivery systems will grow in coming years; therefore, we could expect a remarkable increase in the broad range of materials used for dsRNA delivery. This innovative RNAi delivery method was firstly developed for human therapeutics, and now nanotechnology is being translated to crop protection as a sustainable strategy for pest management, minimizing the impact in the environment and reducing the use of chemical pesticides.

6. Concluding remarks

Regulations for the registration and introduction of nano-agrochemicals into the market are still missing. Uniform worldwide rules for defining nano-agrochemicals and for harmonizing the risk assessment methods are needed.[119] If the rules were based only on particle size, as in the case of the EC recommendations, many recent so-called nanoformulations would be excluded. Conversely, many products on the market for decades without posing particular problems (e.g., microemulsions, formulants such as clays and polymers) would be “suddenly” considered nanomaterials. Moreover, the regulation of a formulation should rely on a science-based assessment of new risks and benefits involved, not only in terms of individual ingredients, but also based on how the whole nano-formulation behaves in the environment. Indeed, such products have the potential to support better management of agricultural inputs and, thus, to reduce the impact of modern agriculture. Additionally, potential risks derived from nanomaterial exposure should be assessed using an appropriately tailored life-cycle perspective. This means taking into account all the phases in which nano-formulations may be found, from the application into the field, potential incorporation into food supply, to the disposal or re-use of the products together with possible influences exerted by peculiar agro-system conditions that may all affect nanomaterial hazardous properties and risk characterization. For instance, the improved bioavailability of a nanopesticide may affect its environmental fate, as well as its toxicity or behavior once absorbed by organisms. Therefore, a robust toxicological assessment of the potential risks associated with the use of nanopesticides, both as nano-formulations of traditional active ingredients or nanomaterials that exhibit pesticide activity, should be performed. The scientific community can positively or negatively affect public opinion on nano-agrochemicals, depending on whether a positive image of the technology (green, smart and safe technologies) is provided or the potential risks are stressed. The purpose of achieving sustainable agriculture overlaps the need for the development of a “green nanotechnology”, a conceptual approach to balance the benefits provided by nano-products in solving environmental challenges with the assessment and management of environmental, health, and safety risks potentially posed by nanoscale materials. However, to be really ‘sustainable’ not only the safety and risks of the final product should be taken in consideration, but also the whole process for its production (e.g. costs, environmental impact, renewability of all the material used for its synthesis/production). A recent analysis of the literature shows that the comparison studies between nano and conventional agrochemicals is insufficient to assess the true gains in agrochemical efficacy from nano-enabled products.[120] Comparisons between nanoformulations and AIs can explain changes in AI behaviour. Comparisons with conventional formulations are necessary to show improved performance and competitiveness against existing products. Thus, three-way comparisons (nano-, conventional formulated products and AIs) would be strongly recommended in future research. The future of nanoagrochemicals may follow two different scenarios.[121] In the first, nanoagrochemicals may be considered as emerging contaminants and the development of the technology will remain limited. In the second, the establishment of highly collaborative and interdisciplinary research could provide fair assessment of both risk and benefits, allowing the deep exploration of nano-agrochemical potential.[120] Focused studies on safe nanotechnology for improving natural agrochemicals could be an attractive green strategy.

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Legend to Figures

Figure 1. Some of the most important natural polymers used for the synthesis of nanocarriers and their source of origin.

Table 1 - Use of natural compounds as agrochemicals: the two sides of the same coin (inspired by table 1 in Dayan et al, 2012[5])

Expected advantages	**Possible constraints**
New and unusual structures	Chemical structures too complex
New sites and mechanisms of action	Unwanted activities against not-target organisms
Structures optimized for bioactivity	Physic-chemical properties not suited for the commercial/applicative needs
Eco-friendly products	Half-life too short
Expectations to be used at low doses	Low effectiveness
Obtainable from living organisms	Not suitable for industrial scaling-up
Extractable from renewable resources	Low yields/High costs of extraction
Faster and simpler screening and discovery procedures	Re-evaluation and production of already known compounds too expensive or not marketable
Higher acceptability by the public opinion	Registration procedures similar to chemicals
Possible lower registration costs	Limited intellectual protection

Table 2 - Some possible advantages of nanotechnology use to overcome natural agrochemical weaknesses (examples in the review and in the provided references)

Natural agrochemical weakness	**Nanotechnology improvement**
Solubility	Favour the solubility of low-soluble natural compounds
Ecological friendliness	Reduce or avoid the use of organic solvents for agrochemical delivery
Bioavailability	Modulate/slow down the release of the compound against the target/in the environment
Dose	Minimize/optimize effective doses
Mobility	Reduce the risks of leaching or volatilization
Target selection	Help the compound to selectively recognize/attack the target
Shelf-life	Preserve from degradation due to biotic and abiotic agents
Adhesion/penetration	Favour the stick on, or the penetration through plant or target surface
Non-target effects	Reduce the toxicity to non-target organisms