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Collection and review of information on nanomaterial-based and nano-enabled plant protection products, biocidal products and fertilising products

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Abstract

This work, under the European Union Observatory for Nanomaterials (EUON), evaluates nanomaterial applications in biocidal, plant protection, and fertilising products for agriculture. It addresses knowledge gaps on applications, exposure and hazards of nanomaterials and nano-agrochemicals, as well as advantages of using them, highlighting the transition to nanomaterial-based and nano-enabled alternatives. An extensive literature search found 3,052 relevant documents from databases with scientific literature and grey literature, assessing nano-agrochemicals' market presence and their comparative efficacy and safety. The analysis of these documents found that nano-agrochemicals often offer enhanced performance, suggesting a shift towards sustainable agriculture. However, existing regulation, notably the Plant Protection Products Regulation (EC) No 1107/2009 and Fertilising Products Regulation (EU) 2019/1009, lack specific provisions for nanoforms, unlike the Biocidal Products Regulation (EU) 528/2012. Recommendations include updating legislation to incorporate nano-agrochemical definition and considerations, establishing a framework for standardized use instructions, creating an EU-level nano-agrochemicals database, and implementing a notification system for manufacturers. Systematic literature reviews and mandatory toxicity and ecotoxicity tests are advised to support efficacy claims and understand regulatory impacts. The present report is meant to ensure broad awareness of nanomaterials safety and utilization in agriculture, aiming for a balance between productivity and minimal health and environmental impacts.

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Key words: Extensive literature search, nanomaterials, agrochemicals, plant protection products, biocides, fertilisers, risk assessments

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Executive summary

This report details a comprehensive study aimed at improving the transparency and understanding of the safety and market dynamics of nanomaterials, particularly in the context of biocidal, plant protection, and fertilising products utilized in agriculture. This study addresses knowledge gaps in the applications, exposure, hazards, and environmental and health impacts of manufactured nanomaterials. It specifically examines how nanotechnology is being increasingly used in various sectors, replacing traditional formulations and products with nano-enabled alternatives.

Objective 1 – Structure literature review – data collection

The primary objective was to perform an extensive literature search (ELS) literature and documentary review of nanoforms of substances and their delivery systems used in agricultural products, evaluating their claimed benefits against potential safety concerns. This analysis also aimed to identify the difficulties and constraints in testing these products that are enhanced with nanotechnology. Aligned with the objectives of the European Commission's Green Deal, specifically the aim to decrease the utilisation and hazards of chemical pesticides by 50% by 2030, this study examines the preparedness of existing regulatory frameworks in addressing the potential risks posed by these nano-formulated substances. A tailored search strategy was employed to retrieve studies, including reviews and grey literature, pertinent to the identification of the nanoforms of (active) substances used in biocidal products, plant protection and/or fertilising products. Additionally, the study covers the delivery systems of the corresponding formulations for agriculture use. The ELS was carried out using the protocol devised in Objective 1. Three different databases were interrogated, namely, PubMed, Web of Science and SciFinder. In parallel, a comprehensive literature search specific for grey documents from multiple data sources, including regulatory agencies and other authorities was also performed. The cumulative search allowed to identify 10,901 documents: 10,696 via databases and registers and 205 from web search engine queries (e.g. Google, Bing, Ecosia) and direct consultations of international authorities (e.g. EFSA, ECHA and FDA). A screening of titles, abstracts and full text for relevance to the risk and safety assessment was performed taking into account inclusion and exclusion criteria. References were analysed by keeping into account the review question. The analysis allowed to retrieve a total number of 3,052 relevant documents. A file with relevant documents (RIS file) is provided and a summarizing table is reported in Annex II.

Objective 2 – Analysis of collected data

The term nano-agrochemical refers to agricultural chemicals containing nanomaterials, typically with external dimensions less than 100nm. The ELS offered insights into the presence of nano-agrochemicals on the global market, as well as a comparative analysis of formulations containing nanomaterials versus conventional products. The review encompassed a wide range of sources, including peer-reviewed scientific articles, reports from research projects, input from regulatory bodies, and feedback from research and industry surveys. This comprehensive approach facilitated a nuanced understanding of the eco-toxicological characteristics, functional variations, and the overarching influence of nanoforms in agricultural applications.

Identified nano-agrochemicals

The ELS enabled the discovery that nano-agrochemicals exhibit enhanced performance characteristics, such as increased efficacy, targeted delivery, and reduced environmental impact compared to their conventional counterparts. Both green synthesized and chemically synthesized nanomaterials are used in various applications, including plant growth and development, crop improvement, photosynthesis enhancement, fertilizers, pest control, and post-harvest technology. For example, nanoparticles, nano-capsules, and nanofibers are used as vehicles to carry and release foreign DNA into plant cells, improving genetic modifications for better crop quality and production. Specific examples of nano-agrochemicals include:

- Carbon nanotubes (CNTs) that penetrate plant cell walls and membranes, aiding in seed germination and growth rates in crops like *Lycopersicon esculentum*.
- Silver nanoparticles (AgNPs) used as growth enhancers in plants like *Bacopa monnieri*, showing growth improvement without apparent toxic effects.
- Titanium dioxide nanoparticles (nTiO₂) that improve plant growth and resistance in saline conditions, enhance photosynthesis, and reduce disease severity in crops like broad beans and corn.
- Mesoporous silica nanoparticles (MSN) that carry DNA and chemicals into plant cells, aiding gene expression and plant genetic transformation.

Despite the promising applications, very few nano-enabled agro-inputs have made it to the market. While nano-agrochemicals offer significant advancements, they also pose potential safety concerns. Studies have shown that metallic nanoparticles can cause oxidative stress in plants, interfering with the electron transport chain and detoxification processes. These effects could lead to genotoxicity and other adverse outcomes. Therefore, there is an ongoing need for comprehensive risk assessments and regulatory guidelines to ensure the safe use of nano-agrochemicals.

Analysis of current practices and regulatory frameworks

The ELS provided significant insights into the safety of nanoform substances and products containing nanomaterials. It revealed gaps within existing legislation, notably the absence of specific provisions addressing nanoforms in the Plant Protection Products Regulation (EC) No 1107/2009 (PPPR) and the Fertilising Products Regulation (EU) 2019/1009 (FPR) while in the Biocidal Products Regulation (EU) 528/2012 nanomaterials are specifically addressed but agricultural uses of active substances is outside the scope of this regulation. Nevertheless, the Biocidal Products Regulation stands out for its explicit mention in Recital (66) of the scientific uncertainty surrounding the impact of nanomaterials on health and the environment. The regulation emphasizes that the approval of an active substance does not implicitly cover its nanomaterial form, highlighting the necessity for explicit mention and regular reviews of nanomaterial provisions to adapt to scientific progress. Introducing specific definitions and frameworks for nano-agrochemicals in existing regulations is crucial for ensuring safety and transparency. For example, establishing a specific register to report information about nano-agrochemical products would create transparency in the supply chain for all organizations and stakeholders involved. Such initiatives would refine the regulation's scope and enhance its effectiveness in addressing the challenges posed by nano-agrochemicals. It is emphasized that the Plant Protection Products Regulation and the Fertilising Products Regulation lack specificity regarding nano-agrochemicals, indicating a pressing need for their evolution. Drawing parallels from the Biocidal Products Regulation, it is evident that introducing a specific definition for nano-agrochemicals, mandating clear labelling of products containing nanomaterials, and conducting detailed risk assessments are critical steps towards ensuring the safety and efficacy of nano-enabled agricultural products. The ELS highlights the potential for nanotechnology to significantly enhance agricultural practices, as nano-agrochemicals can deliver active substances more efficiently and with reduced environmental impact. However, the effectiveness and safety of these nano-enabled products depend on manufactures adhering to specific regulatory frameworks that provide appropriate standard operating procedures, including thorough characterization, to address their unique properties and potential risks.

Recommendations to improve the quantity and quality of information

The study strongly advocates for enhancing tracking and validation technologies for both current and next-generation nano-agrochemicals. This approach aims to improve the identification, safety assessment, and overall information quality regarding nano-agrochemicals, considering that emerging materials may introduce novel risks. Establishing clear preparatory rules and control procedures for nanomaterial characteristics from the outset may mitigate potential issues, though regulatory guidelines will need to be periodic updates as new developments arise and insights are gained.

Among the advanced solutions, the utilization of nano-sensors for precise nanomaterial detection is expected to be particularly promising in the near future. These technological advancements aim to boost the efficiency and safety of nano-agrochemicals in agriculture, contributing to more sustainable and effective farming practices. Additionally, the development of biodegradable nanocarriers and the exploration of novel nanomaterials with reduced environmental and health risks are recommended. This focus on sustainability and environmental compatibility reflects an increasing awareness of the importance of eco-friendly innovations in the nano-agrochemical sector.

In response to the identified regulatory gaps, particularly in the Plant Protection Products Regulation and the Fertilising Products Regulation, which currently overlook the specific challenges posed by nano-agrochemicals, comprehensive updates are proposed. These updates would integrate detailed considerations for nanocarriers, acknowledging the significant role and potential risks associated with nano-agrochemicals, especially regarding toxicity and ecotoxicity concerns. A systematic revision of these regulations is recommended to ensure that nanomaterial-containing substances and products are accurately identified and assessed for safety, aligning with the dynamic advancements in agricultural and biocidal product development and application.

Furthermore, the establishment of a uniform framework for standardized instructions for nano-agrochemical use is emphasized. This framework would require manufacturers to provide detailed usage instructions, accommodating various field application scenarios and ensuring that downstream users, such as farmers and agriscience professionals, have access to clear and authoritative guidance. The approval of these instructions by competent national or EU authorities before market entry is crucial for safeguarding against potential risks and enhancing regulatory oversight.

The creation of a specialized database of nano-agrochemicals at the EU level, curated by the European Union Observatory for Nanomaterials (EUON) and potentially in collaboration with the European Food Safety Authority (EFSA), is proposed. This initiative aims to centralize critical information on nano-agrochemicals, facilitating access for regulators, researchers, and stakeholders. The database would categorize nano-agrochemicals based on their intended use, chemical nature, and regulatory compliance, significantly streamlining the regulatory process and promoting safer, more effective nano-agrochemical application across the EU. Additionally, a comprehensive notification system for manufacturers and importers is suggested, similar to the EU Common Entry Gate (EU-CEG) framework used in other regulatory contexts. This system would ensure that all nano-agrochemical products entering the EU market are registered, and their safety and compliance information is transparent and accessible.

Systematic literature reviews to support the efficacy claims of nano-enabled versus conventional agrochemicals are deemed essential. Despite potential data gaps, especially in toxicity and ecotoxicity assessments, these reviews are crucial for highlighting the advancements offered by nano-agrochemicals. Market authorization for new nano-agrochemical products should hinge on demonstrating a distinct advantage over existing solutions, reinforcing the principles of safety, sustainability, and enhanced productivity. Lastly, mandating the submission of comprehensive toxicity and ecotoxicity test results is highlighted as a critical step towards understanding and regulating nano-agrochemicals. With the multifaceted nature of their (eco)toxicological profiles, a broad spectrum of standardized and validated testing methodologies is necessary.

This holistic evaluation approach will ensure rigorous environmental and health safety assessments, underpinning informed regulatory decisions and protecting ecological integrity and human health. Through these recommendations, the study lays a foundation for a nuanced understanding and regulation of nano-agrochemicals, aiming to balance agricultural productivity with minimal adverse health and environmental impacts.

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1 Introduction

1.1 Contract references

This contract was commissioned by the European Chemicals Agency (ECHA) to Innovamol Srl to perform an extensive systematic literature review with the aim to collect and review information on nanomaterial-based and nano-enabled plant protection products, biocidal products and fertilising products.

- Contractor: Innovamol Srl
- Members of the team: Daniele Urbani, Ph.D; Martina Evangelisti, BSc; Camilla Bebi, BSc; Marco Daniele Parenti, Ph.D, Greta Varchi, Ph.D, Alberto Del Rio, Ph.D.
- Tender reference: ECHA/2022/512

1.2 Background information

The European Union Observatory for Nanomaterials (EUON) aims to improve the transparency of information on the safety and markets of nanomaterials. One of the main ways to achieve this objective is by running studies to address important knowledge gaps on nanomaterials.

Biocidal products, as well as plant protection products and fertilising products for agricultural use, availing from nanotechnology, have been the subject of many research and development (R&D) studies over the years. A move from conventional products towards the use of nanomaterial-containing formulations or nano-enabled formulations (e.g., with products encapsulated in nanosized delivery systems) is observed. However, little is known about the current and emerging applications of manufactured nanomaterials in such products, as well as on their resulting exposure to and possible effects on human health and the environment.

The application of nanotechnology (use of nanoforms of substances and formulations containing nano-enabled substances) offers new opportunities to increase efficacy and efficiency overcoming some of the issues related to dosing and applying the products containing active substances and fertilising products. They enable target-specific, precise, and slow(er) release of the product, reduced use of active substances, ease of application, reduced risk for non-target organisms and operator exposure among others. Therefore, this specific study can also be seen in the context of the compliance with the Green Deal goals: the European Commission proposes to reduce the use and risk of chemical pesticides by 50% by 2030 in the EU. However, the uncertainties associated with the potential adverse effects of some of these nanomaterials and their delivery systems require further consideration.

1.3 Objectives as provided by ECHA

The aim of the contracted study was to conduct a thorough, systematic and critical literature and documentary review on nanoforms of (active)substances used in biocidal products, plant protection products and/or fertilising products. Additionally, the study shall cover the delivery systems of the corresponding formulations for agriculture use. All collected data shall be critically analysed and organised. The literature review shall be based on information available from public sources, including peer-reviewed scientific literature, literature reviews on the topic if available, publicly available reports from e.g., research projects, agencies, and regulatory bodies.

The study shall assess what general conclusions can be made regarding the claimed benefits and the potential safety concerns associated with nano-enabled plant protection products, biocidal products and fertilising products and what challenges and pitfalls have been identified as surrounding the testing of these products.

The review shall evaluate and discuss the adequacy of the current practices and the preparedness of regulatory frameworks to ensure the identification and risk assessment of these substances and products. Finally, the study shall explain how the outcome of the review could be used to change policy instruments.

2 Data and methodologies

This chapter describes the definition of all the elements of the protocol for the systematic review that were adopted during the execution of the work. All aspects were discussed and agreed with ECHA before proceeding to the actual searches.

2.1 Structured literature review

The initial work was focused on the development of the protocol for the systematic review, according to EFSA guidelines and the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, discussed in section 3.1.4) on systematic reviews, for the retrieval of science-based evidence on nano-agrochemicals (nanostructured agricultural chemicals) used as plant protection product, biocidal and fertiliser.

2.1.1 Protocol for the systematic review

The protocol for the systematic literature review followed the EFSA guidance on systematic reviews (EFSA Guidance for those carrying out systematic reviews European Food Safety Authority, 2010) and the PRISMA statement on systematic reviews (Moher *et al.*, 2009). It has been registered on the international prospective register of systematic reviews PROSPERO ([CRD42023482659](https://doi.org/10.1186/1745-7189-4-2659)). The following elements of the systematic review were defined:

- Review question: Which are the benefits and the potential safety concerns associated with nano-enabled plant protection products, biocidal products and fertilising products?
- PI/ECO, i.e., population (P), intervention/exposure (I/E), comparators (C) and outcomes (O). The definition of PI/ECO was essential to identify the eligibility (inclusion and exclusion) criteria. The following table describes the adopted definition.

TABLE 1 – PICO AND PECO DEFINITION.

	Description
P	Populations (P) will identify any cell-based living system, including cell-lines, animals, and humans. This broad definition is appropriate for considering all possible studies related to the potential adverse effects of these substances, even beyond those strictly related to adverse effects in humans.
I/E	Interventions and exposure (I and E) will identify any intervention and/or exposure to which the population is exposed by any of the nanoforms of (active) substances used in biocidal products, plant protection products, and/or fertilizing products.
C	Comparators (C) will identify control or reference groups in experimental studies or documents not exposed to I or E and information on regulatory documents. For the scope of this tender, we propose using conventional products without nanoforms as comparators.
O	Outcomes (O) will identify: <ol style="list-style-type: none"> adverse effects of nanoforms of (active) substances used in biocidal products, plant protection products, and/or fertilizing products on humans and/or animals relating to acute toxicity, repeated-dose toxicity, mutagenicity, carcinogenicity, reproductive or developmental toxicity; adverse effects of these substances on humans and/or animals resulting from different routes of exposure (e.g. inhalation, dermal, ingestion, intravenous and other parenteral administration routes) with association of specific material parameters like dimensions, shape, functionalization, surface chemistry, physicochemical properties, preparation methodology; adverse effects of these substances in cells that would impact normal and physiological cell proliferation and development (e.g. cytotoxicity, apoptosis, necrosis, migration, anoikis, differentiation); in silico, in vitro or in vivo methods applied to these substances for assessing any of the adverse effects described in the previous points.

- Keywords and query syntax for the selected databases, which were grouped in three blocks relevant to different variables of the search question:
 - Block 1: keywords identifying the nano-morphology of the material;
 - Block 2: keywords (i) identifying the typology of plant protection products, biocides or fertiliser that may occur;
 - Block 3: keywords identifying generic elements related to farming and toxicology aspects.

The structure of the query search combined the keywords in the three blocks by using the Boolean operator “AND” (see Figure 1).

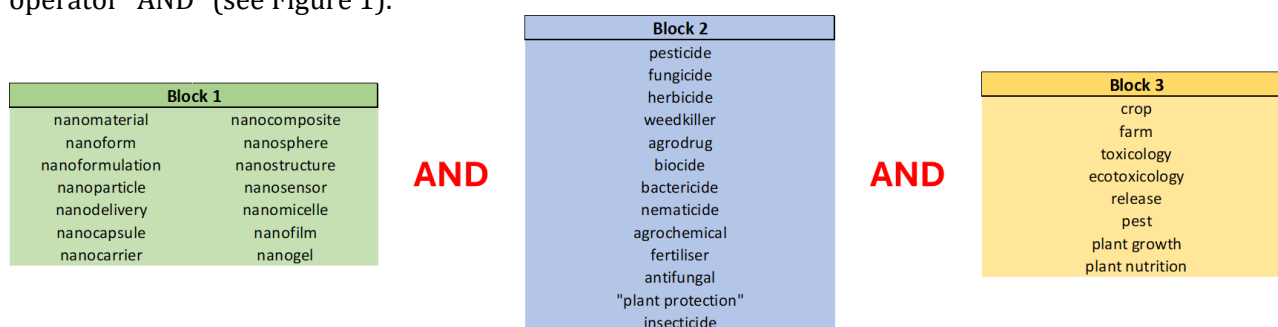


FIGURE 1 – GENERAL STRUCTURE OF THE QUERY SEARCH.

- Search strings were written using the selected keywords, the query syntax and Boolean operators. All the databases and data sources were able to support automated queries. Searches were performed with Boolean operators “AND” and “OR” and parenthesis combinations involving keywords or chemical structures. The Boolean operators “NOT” was not used for queries in order to avoid automatic rejection of potentially relevant documents.
- Inclusion and exclusion criteria for abstract and title screening, and for full-text screening, based on the PI/ECO definition.

As a general rule, any document referring to a nanomaterial or combination of nanomaterials used in the agronomical field that has at least one dimension below 100 nm and act differently from the non-nanostructured material were included as relevant.

To account in an efficient and unambiguous manner the reasons for excluding a particular document, a scheme to encode exclusion criteria (EC) was devised and is summarised in Table 2. Exclusion codes represent the different reasons why a specific document was excluded.

TABLE 2 – EXCLUSION CRITERIA.

Exclusion code	Description of exclusion criteria
EC1	Documents describing nanofarm preparation, manufacturing, and/or applications with no association with any kind of toxicological or functional data.
EC2	Articles describing toxicological or functional data in cells, animals, or humans that are not test-item related (i.e., no clear link with nanofarms of active substances).
EC3	Documents describing toxicological or functional data of materials that are not nanofarms of active substances used in biocidal products, plant protection products, and/or fertilizing products in the agrochemical field.
EC4	Documents with general speculation, general description, or historical description of nanofarms of active substances.
EC5	Any other documents that cannot be categorized in inclusion criteria and cannot be excluded with EC1-EC4 codes.

- Screening and reporting methodology and data extraction tables were discussed in depth with ECHA. Articles were divided by area of interest, covering different features of the nanomaterials and populations included within the PICO/PECO definitions as described in the section below.

2.1.2 Search strategy

The databases used for the systematic literature search were PubMed, a biomedical database, the core collection of Web of Science (WoS_CC), a multidisciplinary bibliographic database and SciFinder, a multidisciplinary but chemicals-oriented database. Tailored searches were conducted for each database due to their intrinsic differences. The query search was run in SciFinder on the 07th September 2023 and on PubMed and WoS_CC on the 13th September 2023 as reported in Table 3.

TABLE 3 – PUBMED, WOS_CC AND SCIFINDER QUERIES.

Database	Queries
PubMed	<i>(nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (pesticide OR fungicide OR herbicide OR weedkillers OR agrodrug OR biocide OR bactericide OR nematocide OR agrochemical OR fertiliser OR antifungal OR “plant protection” OR insecticide) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)</i>
WoS_CC	<i>TS=((nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (pesticide OR fungicide OR herbicide OR weedkillers OR agrodrug OR biocide OR bactericide OR nematocide OR agrochemical OR fertiliser OR antifungal OR “plant protection” OR insecticide) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition))</i>
SciFinder	<i>(CAS numbers divided by OR) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)</i>

Even though all the queries utilize the same set of keywords, they differ in syntax due to the fact that PubMed and WoS_CC utilize distinct search engines. The SciFinder query was designed such that the CAS numbers of pertinent substances are utilized in box 1 to direct the search towards the appropriate subject matter.

Due to the 2000-character constraint of the SciFinder query search field, it was not possible to search for every substance in a single query. Consequently, a strategy involving multiple queries was adopted. Each query that was employed to conduct this database search is detailed in Annex I. The CAS numbers utilized for the query search in SciFinder were acquired through a review of online databases such as [ECHA fertiliser regulation](#), [ECHA list of biocide active substances](#), [European Commission list of active pesticides](#) and the [Registro de Productos Fitosanitarios by the Ministerio de Agricultura, Pesca y Alimentación](#). The corresponding information is provided in Annex I Section 8.1.

There were no limitations imposed on the publication period during the research processes. Articles in languages other than English were discarded.

The raw data were obtained by exporting references in NBIB or RIS formats from PubMed, WoS_CC, and SciFinder. The references were managed using Zotero V. 6.0.13, whereas the generation of RIS and CSV files was accomplished using Python scripts developed in-house. The InnoLiterature® database version V.1.0 (Innovamol Srl, 2023) was utilized for data fusion and relevance selection. The report was composed using Microsoft Word and Excel, supplying both text and table formats.

Post-processing was performed on the repository of documents obtained to accomplish the following:

- i) merge data from the various searches;
- ii) eliminate duplicates;
- iii) verify the integrity of each entry; and
- iv) rectify reference citations as necessary.

The post-processing operation yielded a non-redundant list of entries, which was subsequently exported to Excel. In Excel, custom variables were incorporated to correspond with the exclusion criteria code (EC) and area of interest.

2.1.3 Grey literature

A protocol for the grey literature search was implemented to recover the maximum amount of non redundant relevant grey literature documents and information from Web pages. A specific search on the main Web search engines (Google, Bing and Ecosia) was ran and a specific search on the Web page of international authorities was executed to grasp any additional document that was missed by the white literature search. All the grey literature search were run on the 17/01/2024. The complete list of the Web sites checked, and the relative query structures are reported in Table 4.

TABLE 4 - GREY LITERATURE SEARCHES

Repository	URL	Query	Filter applied
Google	www.google.com	<i>nano agrochemicals efficiency and toxicity</i>	/
Bing	www.bing.com	<i>nano agrochemicals efficiency and toxicity</i>	/
Ecosia	www.ecosia.org	<i>nano agrochemicals efficiency and toxicity</i>	/
FDA	https://www.fda.gov	<i>nanomaterial agriculture</i>	/
ECHA	https://www.echa.europa.eu	<i>nanomaterial agriculture</i>	/
EFSA	https://www.efsa.europa.eu/en	<i>nanomaterial agriculture</i>	Filter by type with only Consultation, Corporate documents and Scientific outputs
WHO	https://www.who.int	<i>nanomaterial agriculture</i>	/
FAO	https://www.fao.org/home/en	<i>nanomaterial agriculture</i>	/
FSA	https://www.food.gov.uk	<i>nanomaterial agriculture</i>	/
EFFA	https://www.effa.eu	<i>nanomaterial</i>	/

EPA	https://www.epa.gov	<i>nanomaterial agriculture</i>	Filtered by resource type with only reports and assessments
Health Canada	https://www.canada.ca/en/health-canada.html	<i>nanomaterial agriculture</i>	/
FSANZ	https://www.foodstandards.gov.au	<i>nanomaterial agriculture</i>	Filtered out by type with only publication
China National Medical Products Administration	http://english.nmpa.gov.cn/	<i>nanomaterial agriculture</i>	/
OECD	https://www.oecd.org	<i>nanomaterial agriculture</i>	/
IFOAM	https://www.ifoam.bio	<i>nanomaterial agriculture</i>	Filtered out by type with only publication

For Google, Bing and Ecosia only the first 50 references were checked to assess if any relevant grey literature were retrieved by the query run. While searching for the grey literature in the various international authorities' websites only the first 20 documents for each search were actually checked to assess if any relevant grey literature documents were retrieved by the query run. The results obtained from the grey literature search are reported in section below.

2.1.4 Title and abstract screening

The documents retrieved from PubMed, WoS_CC, SciFinder and the grey literature were exported to the InnoLiterature® database, where the screening of titles and abstracts for relevance was performed by the team experts keeping into account inclusion and exclusion criteria. Full-text examination was performed for all relevant documents selected after title and abstract screening and for those on which a decision could not be taken after title and abstract screening, as described in section below.

The work was organised so that two experts, with different expertise, independently reviewed the documents, especially in case of borderline documents¹. A decision on the relevance was made for each record/document if at least one expert judged it relevant to the specific inclusion and exclusion criteria. For each document, the following actions were performed:

- The relevance of each document was evaluated by checking if relevant keywords described a real scientific relationship between the nanomaterial and the agricultural area of usage,
- The relevance of each document was evaluated against inclusion criteria,
- The relevance of each document was evaluated against exclusion criteria.

In many cases, the documents were judged clearly irrelevant and excluded without further actions. In other cases, the analysis of the title and abstract were sufficient to judge the document relevant. In this case, documents were labelled as relevant, and no further action was performed at this stage. Full-text examination was performed for all relevant studies selected after title and abstract screening and for those on which a decision could not be taken after title and abstract screening. It is noteworthy that, in case of borderline documents, a conservative approach was used to ensure that the document was not discarded and evaluated in a subsequent step.

2.1.5 Full text screening

For records that passed the relevance screening based on titles and abstracts (or, in cases when a final decision could not be made based on the title and/or abstract alone), the full paper was screened. Open

¹ Expert 1: expert in nanomaterials with at least 3 years of experience; Expert 2: expert in toxicology with at least 3 years of experience; Expert 3: expert in risk assessment methodologies with at least 3 years of experience.

access articles or articles available from affiliations of team members were downloaded. Having at disposal the pdf files of relevant articles and the list of relevant keywords and the inclusion and exclusion criteria, the two experts that performed the title/abstract screening and, if needed, the third reviewer as well, screened the full text for relevance and thereafter proceeded with the data extraction.

2.2 Analysis of the collected data

2.2.1 Data extraction

The data extraction was conducted as follows:

- Number of reviewers: one for the data extraction and another one for the validation of the extracted data.
- Disagreements: in case of borderline data to be extracted, a conservative approach was used to ensure that no information was lost. Data was checked by a third team member for confirmation.
- Missing data: missing information/data resulted in a “/” symbol in each cell under the corresponding extraction field where the data is missing.
- Tool for recording data: Microsoft Excel
- Other data extraction and management tools: ABBYY fine reader V15 for extraction of the text and InnoLiterature® database V.1.0 for extraction of bibliographic information.

That information is reported in the Excel summary table enclosed to this report in Annex II (Annex II - Summarizing table - nanomaterial-based and nano-enabled plant protection products, biocidal products and fertilising products.xlsx).

2.2.2 Summarizing tables structure

Within this task, relevant and critically assessed articles were divided by area(s) of interest, covering different characteristic of the nanoparticles discussed in the documents and requirement included in the PICO/PECO definitions. This approach was the basis to annotate and extract specific high-level information, including:

- Bibliographic information:
 - Title
 - Abstract
 - Author
 - DOI text
 - DOI URL
 - Item Type
 - Journal
 - Language
 - Pages
 - Volume
 - Year
 - Comments
- Area of interest (checkbox - multiple choices possible):
 - A1: General information on nanomaterials containing an active compound;
 - A2: Marketing in the EU;
 - A3: Information on (eco)toxicological properties;
 - A4: Information on function and efficiency;
 - A5: Potential health risks for workers, farmers and consumers.
- High level information (textbox - multiple data entering possible):
 - Nanoparticles (NPs) material;
 - Active substance;
 - NPs size & morphology;
 - Efficiency improvements;
 - (Eco)toxicological properties.

2.3 Survey structure

A survey to collect information from experts, stakeholders and other personas has been launched between the 21st of November 2023 and the 29th of February 2024. The online survey was created with Microsoft Team Forms and links were disseminated in institutional and company webpages as well as LinkedIn pages, targeted mailing lists and personal messages to stakeholders. The survey was structured in 6 different questions written as reported in Table 5.

TABLE 5 - SURVEY QUESTIONS

Question number	Question text	Possible answers
#1	Please select from the list below your occupation or the field in which your organisation is involved	<ul style="list-style-type: none"> Researcher Farmer Chemical manufacturer Regulatory agency/consultant Other (open-handed answer)
#2	Which areas are you presently exploring in connection with nanostructured agrochemicals?	<ul style="list-style-type: none"> Pesticide Fertiliser Fungicide Herbicide Bactericide Virucide Nematicide Salt tolerance enhancer Soil quality enhancer Metal chelator Not involved/not interested Other (open-handed answer)
#3	Can you indicate what is in our opinion the primary application(s) or area(s) of research or use for nanostructured agrochemicals?	<ul style="list-style-type: none"> Crop protection Soil improvement Pest control Environmental protection Other (open-handed answer)
#4	Based on your knowledge or experience, what do you perceive as the key benefits or advantages of using nanostructured agrochemicals and related fields?	<ul style="list-style-type: none"> Reduce the amount of chemical used Improve the stability of the agrochemical over time Reduce costs (e.g. less labour costs) Reduce the number of applications of agrochemicals Improve the efficacy of treatment Improve or reduce the water solubility of the agrochemical Ensure better safety of workers Improve the safety of food/feed products Reduce the volatility of agrochemicals Other (open-handed answer)
#5	Similarly, based on your knowledge or experience, what are the primary concerns or challenges associated with the use	<ul style="list-style-type: none"> Ecotoxicity Human toxicity (workers) Human toxicity (consumers) Higher cost of production Higher cost of food/feed products Negative perceptions about nanostructured chemicals

	of nanostructured agrochemicals and related fields?	Manufacturing and scale-up hurdles Regulatory and/or approval procedure hurdles Other (open-handed answer)
#6	In your opinion, what potential developments, advancements and uses do you foresee in the implementation of nanostructured agrochemicals and related fields?	Open-handed answer

3 ELS results

3.1 Structured literature review

The search strategy was defined with the provision described in the previous sections.

3.1.1 Result from the systematic review

Table 6 shows the results obtained from three different queries and the combination of those with the removal of redundant documents obtained within this project.

TABLE 6 – RESULTS OF THE WHITE LITERATURE QUERY SEARCHES.

Query_date	Number of hits
PubMed_13/09/2023	5,409
WoS_CC_13/09/2023	1,878
SciFinder_07/09/2023	8,474
Total with duplicates	15,761
Total without duplicates	10,696

3.1.2 Grey literature searches results

Table 7 shows the results obtained from grey literature query searches ran following the protocol described in Section 2.1.3. The number of hits obtained are reported without duplicates.

TABLE 7 – RESULTS OF THE GREY LITERATURE QUERY SEARCHES.

Query_date	Number of hits
Google+Bing+Ecosia_17/01/2024	16
FDA_17/01/2024	13
ECHA_17/01/2024	29
EFSA_17/01/2024	13

WHO_17/01/2024	20
FAO_17/01/2024	20
FSA_17/01/2024	1
EFFA_17/01/2024	0
US EPA_17/01/2024	20
Health Canada_17/01/2024	20
FSANZ_17/01/2024	9
China National Medical Products Administration_17/01/2024	0
OECD_17/01/2024	20
IFOAM_17/01/2024	20
Others_17/01/2024	4
Total without duplicates	205

3.1.3 Summary of the relevance assessment

From the title and abstract screening 3,052 document were selected as relevant. Consequently, 7,851 documents were identified as non-relevant according to the review questions and they were excluded from the analysis. The subsequent full-text analysis allowed to discard additional documents, as shown in Table 8. Interestingly, as depicted in Figure 2, most of the documents were identified in the primary literature while 17.7% of documents were relevant reviews, 7.4% were relevant registered patents and only a small fraction consisting in other type of documents.

TABLE 8 - RELEVANT DOCUMENTS AFTER FULL TEXT EXAMINATION.

Documents identification	Number of relevant documents
Research articles	1,321
Reviews	332
Patents	139
Other document types ²	80
Total number of relevant documents	1,872

² Analysis, Book, Comment, Communication, Editorial, Letter, Opinion, Perspective, Protocol, Report, Dissemination Platform, Guideline, Thesis, Web page, Opinion, International authorities report.

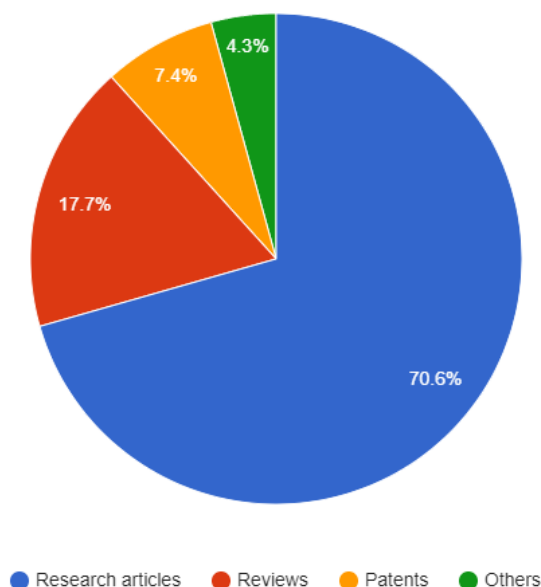


FIGURE 2 – DISTRIBUTION OF RELEVANT DOCUMENTS BY TYPE.

Interestingly, after title and abstract screening and full-text examination, several documents were excluded as the described nano-agrochemicals failed to fit the definition of nanosized material, i.e. size greater than 100 nm, or their field of application was outside the scope of this tender (e.g. pharmacology or medical devices).

It is important to reiterate that, while all type of documents were considered for the extensive literature review to provide a consistent methodology, after the full-text screening and for the purpose of the data extraction, only original research articles were used as the source of data. A classification into area of interest was carried out for original research article and for other type of documents. It is important to note that, since documents might contain multidisciplinary research, the same article can be assigned to multiple area of interest.

As depicted in Table 9 and illustrated in Figure 3, the bulk of the documents primarily focus on the functionality and efficacy of nano-agrochemicals, often detailing the characteristics of nanomaterials that contain an active compound. Additionally, numerous studies within the dataset specifically address the toxicological and ecotoxicological properties of these materials. A smaller subset of the literature also examines the potential health risks associated with exposure to these nano-agrochemicals, particularly for workers, farmers, and consumers. Notably, the scope of our study did not uncover definitive evidence regarding the actual usage levels of these nano-agrochemicals within the agricultural sector of the EU. This gap underscores the absence of targeted documentation and calls for further investigative efforts to ascertain the prevalence and impact of nano-agrochemical applications in European agriculture. This lack of data highlights a significant area for future research and regulatory attention to ensure safe and informed use of these advanced materials in the agricultural industry.

TABLE 9 – DISTRIBUTION OF RELEVANT RESEARCH ARTICLES ACROSS AREA OF INTEREST.

Area of interest	Information fields and research area	Number of relevant documents
#1	General information on nanomaterials containing an active compound	674
#2	Marketing in the EU	0
#3	Information on (eco)toxicological properties	253
#4	Information on function and efficiency	1244
#5	Potential health risks for workers, farmers and consumers	3

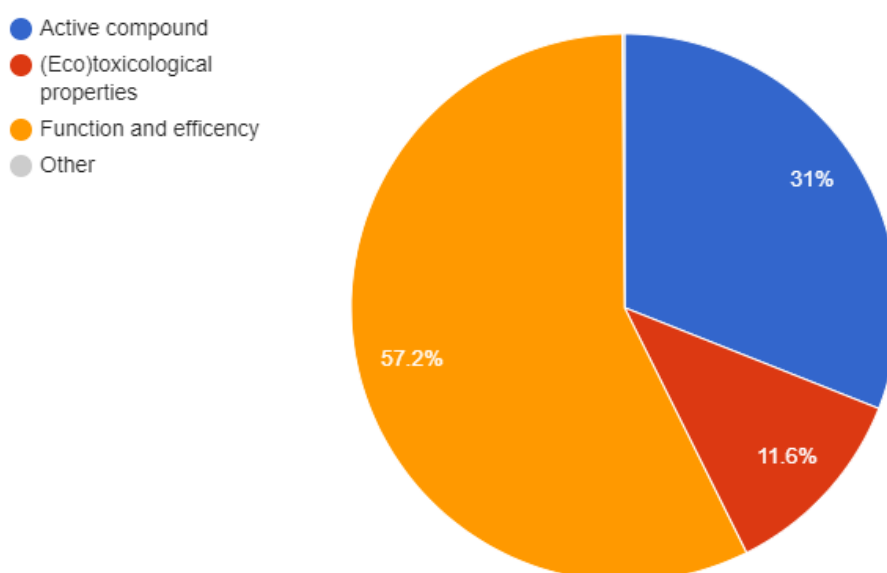


FIGURE 3 – CLASSIFICATION OF RELEVANT RESEARCH ARTICLE BY AREA OF INTEREST.

From the 205 documents labelled as grey literature, only 39 were considered relevant after full text screening but since none of those is a research paper reporting original data, no data extraction was executed. A complete list of relevant grey literature documents is available in Annex II Section 8.2.

3.1.4 PRISMA statement

This section outlines the adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher *et al.*, 2009), a structured framework ensuring the transparent and systematic reporting of reviews. The flowchart exemplifies the methodical approach taken for the identification, screening, and inclusion of studies, reviews, books and other type of articles, highlighting the integrity and robustness of the research process adopted for this call.

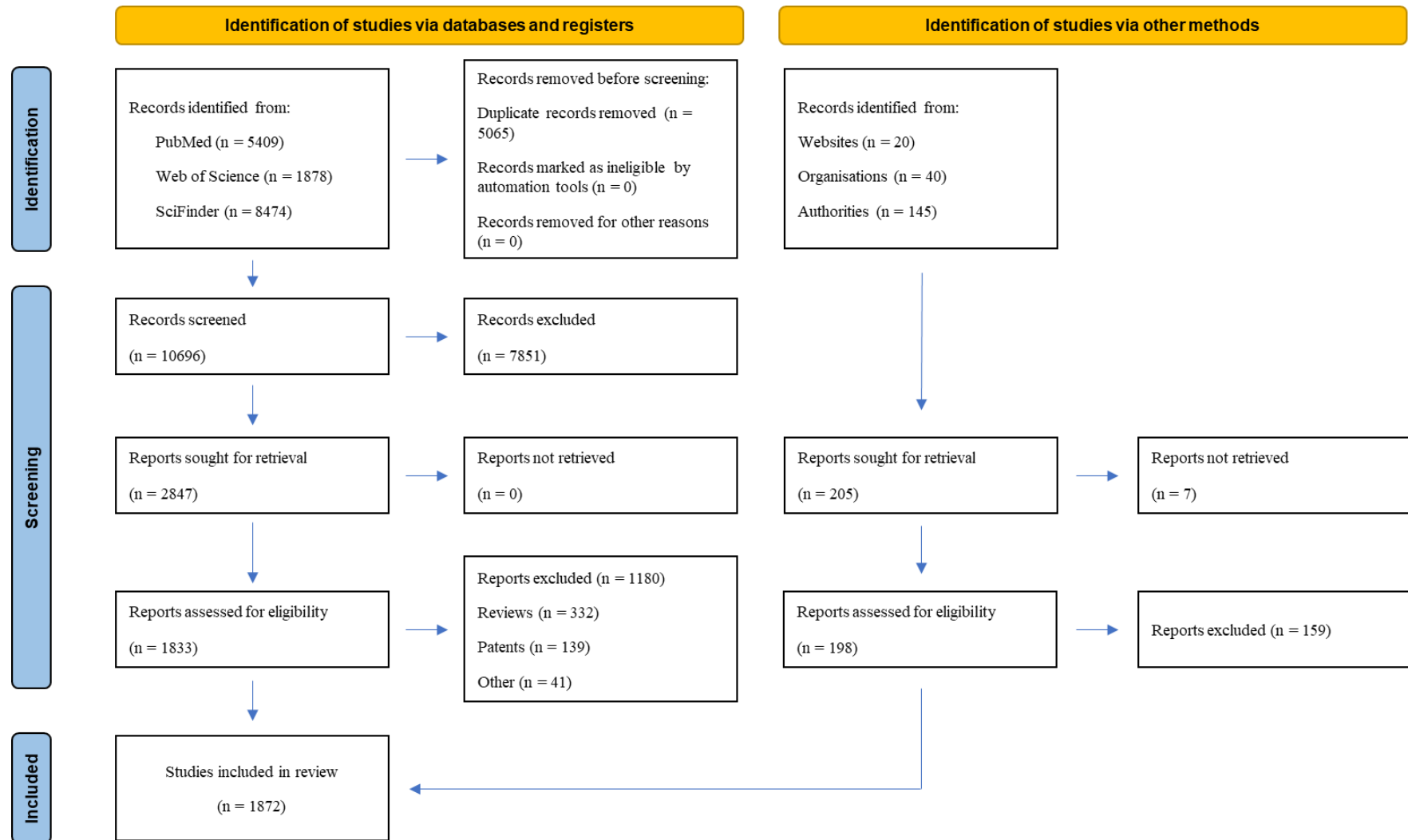


FIGURE 4 – PRISMA - PREFERRED REPORTING ITEMS FOR SYSTEMATIC REVIEWS AND META-ANALYSES - FLOWCHART.

3.2 Analysis of the collected data

The following chapters summarize the information collected during the data extraction. The information regarding the different agrochemical categories (fertilisers, fungicides, etc.) and type of nanoparticles' material for each category is proposed. The full information extracted during the execution of the ELS is reported in the summarizing table reported in Annex II Section 8.2. Table 10 presents the results obtained in terms of number of relevant documents retrieved and summarised in each category.

TABLE 10 – RELEVANT ORIGINAL DOCUMENTS ANALYSED FOR EACH NANO-AGROCHEMICAL CATEGORY

Nano-agrochemical category	Number of relevant documents analysed
Abiotic or biotic stress tolerance enhancer	66
Arachnicide	8
Bactericide	135
Fertiliser	580
Fungicide	318
Herbicide	37
Insecticide	151
Molluscicide	2
Nanocarrier	11
Nematicide	27
Soil quality improver	25
Toxic compound	56
Virucide	12

Since some nano-agrochemicals are used in more than one category the sum of the relevant documents analysed does not match the total number of relevant original documents retrieved (1321). In the following chapters and sub-chapters an insight into the most relevant documents is provided for each agrochemical category identified in Table 9. When several relevant documents were retrieved for each agrochemical category, a sub-chapter division was provided taking into account the nanostructured material of the nano-agrochemical or the inherent quality of a specific category of substances.

A comprehensive access to the extensive data collected can be performed by consulting summarizing tables presented in Annex II, Section 8.2 (see Figure 5). Tables are formatted as an Excel file, designed to serve as a dynamic tool for filtering and retrieving specific information based on the user's needs. Users can effectively navigate through the dataset by applying filters to columns, which include detailed descriptors such as type of nanomaterial, agrochemical category, and dimensionality, among others. Section 8.2 offers a thorough description of the content of each column along with practical tips on how to utilize the Excel file for optimal data extraction and analysis.

The screenshot shows an Excel spreadsheet with the following columns: Title, Abstra, Authr, DOI TE, DOI, DOI Li, Item Ty, Journ, Langua, Page, Volun, Year, Comme, Area of interest, and High level information. The table contains 41 rows of data. A filter menu is open over the table, showing options to sort (Sort A to Z, Sort Z to A) and filter by color. A search box is also visible, containing a list of checked items: Fulvic acid (FvA), fungal extract, Fungicides (hexaconazole and da, Fungus B. tetramera KF934408, Fungus Trichoderma harzianum, Fusarium culmorum strain JTW1, Fusarium solani IOR 825, and garlic essential oil.

FIGURE 5 – PREVIEW OF THE SUMMARIZING AS PRESENTED IN ANNEX II (EXCEL FILE).

3.2.1 Fertilisers

A nano-fertiliser can be defined as a substance acting as a fertiliser, having dimensions in the nanoscale range, and behaving differently from the corresponding bulk substance added directly to the plant or in the soil to supply one or more nutrients required for the healthy growth of plants.

3.2.1.1 Zinc-based fertilisers

Zinc oxide nanoparticles (ZnO-NPs) have a notable capacity to improve plant growth, increase crop yield, and optimise nutrient management, while carefully balancing their positive effects with possible toxicity. The application of ZnO-NPs to various plant species demonstrates a diverse array of impacts, which are determined by factors such as nanoparticle size, coating, concentration, and environmental conditions. ZnO-NPs have a significant effect on the growth of roots and shoots. For example, in pea plants, various compositions of ZnO-NPs were found to either equal or exceed the growth measurements of control samples, indicating their ability to promote plant growth without negatively impacting the weight of the roots (Skiba *et al.*, 2020). Conversely, elevated levels of Zn in soil have shown to be toxic to plants, highlighting the significance of meticulously regulating the rates at which nanoparticles are applied (Moghaddasi *et al.*, 2017).

Applying ZnO-NPs and ZnSO₄-bulk to the leaves of wheat has been demonstrated to effectively increase the zinc content in the grains, while not significantly impacting the crop yield. This suggests that these substances have the potential to be used for enriching crops with vital nutrients. However, these applications did not consistently affect the distribution of other trace elements, indicating that ZnO-NPs have a specific ability to manage nutrients (Sun *et al.*, 2020). The application of ZnO-NPs, either through foliar sprays or soil amendments, has been scientifically proven to enhance crop yield in rice. This method has also shown positive effects on spikelet number, filled grain rate, and overall biomass. These improvements may be attributed to enhanced absorption of nutrients and physiological reactions, such as elevated chlorophyll levels and increased activity of antioxidant enzymes (G. Yang *et al.*, 2021).

Nevertheless, concerns have been raised about the potential toxicity linked to ZnO-NPs. While certain concentrations have been found to have no significant effect on seed germination and vigour, other research suggests that the impact on plant growth and metabolism varies depending on the concentration of ZnO-NPs. High concentrations of ZnO-NPs (150-200 mg/L) were found to inhibit root elongation in peas, indicating potential phytotoxic effects (Srivastav *et al.*, 2021). Moreover, the interplay between ZnO-NPs and micronutrients such as Fe and Cu indicates intricate influences on the dynamics of plant nutrients. This highlights the need for meticulous control of nanoparticle concentrations to minimise detrimental effects on plant well-being and nutrient equilibrium. In particular, a study by Skiba *et al.* (Skiba *et al.*, 2020) emphasises the two-fold function of ZnO-NPs in agriculture, serving as both growth promoters and potential causes of toxicity.

Similarly, zinc nanoparticles (Zn-NPs), i.e. nanoparticles made exclusively by zinc, or zinc-based NPs having also other components, have been found to greatly enhance plant growth, nutrient absorption, and crop yield in a wide range of agricultural plants. Research has shown that there are improvements in shoot and root growth, photosynthetic efficiency, and grain yield as a result of increased phosphorous mobilisation and microbial activity in the rhizosphere (Tarafdar *et al.*, 2014). In addition, the combination of Zn-NPs with lower amounts of chemical fertilisers resulted in enhanced plant measurements, soil quality, and microbial populations, suggesting a cooperative influence that supports sustainable agricultural methods (Nandy, Das and Tarafdar, 2023). Zn-NPs applied as foliar treatments in canola demonstrated a substantial enhancement in protein, amino acid content, and antioxidant enzyme activities. However, the impact on oil content and other phytochemicals varied, indicating that the responses were dependent on the dosage administered (Sohail *et al.*, 2020).

The application of zinc oxide quantum dots (ZnO-QDs) on the leaves of tomato seedlings resulted in improved growth and metabolic activity. This treatment outperformed other zinc-based treatments,

including zinc nano-formulations, in terms of biomass, height, and photosynthetic efficiency. ZnO-QDs specifically enhanced the overall fresh weight and height of the plants by 42.0% and 21.3% respectively. Additionally, they also improved the transpiration rate, indicating an increase in metabolic activity and overall plant vigour. ZnO-QDs notably increased the ratio of chlorophyll a/b and the content of carotenoids, thereby improving the transport of photosynthetic electrons and the absorption of light. Contrary to ZnSO₄, which resulted in oxidative stress, ZnO-QDs did not lead to notable enhancements in antioxidant enzyme activities, suggesting the absence of phytotoxicity. The exceptional efficacy of ZnO-QDs in enhancing plant health and growth, coupled with negligible oxidative stress and optimal nutrient movement, highlights their potential as a valuable nanomaterial in agricultural contexts (Sun *et al.*, 2023).

The study by Iranbakhsh *et al.* (Iranbakhsh *et al.*, 2018) revealed that the application of ZnO-NPs on seedlings led to a decrease in growth and biomass, without inducing necrosis or chlorosis. The detrimental impacts of ZnO-NPs were significantly reduced through plasma treatment, resulting in a notable improvement in plant growth and the content of photosynthetic pigments. This indicates that plasma treatment plays a protective role against the toxicity of ZnO-NPs. In contrast, Pereira *et al.* (Pereira *et al.*, 2020) investigating the effects of ZnO-NPs on microalgae found that the toxicity of these nanoparticles varied depending on their concentration. Lower concentrations (under 10 mg/L of ZnO-NPs) had minimal impact, while higher concentrations (over 10 mg/L of ZnO-NPs) strongly suppressed growth and caused changes in the organism's physical structure. These findings suggest that ZnO-NPs have complex effects on various biological systems, emphasising the potential of plasma treatments in reducing toxicity and the significance of concentration in evaluating the risks of nanotoxicity.

3.2.1.2 Iron-based fertilisers

Recent research has shown that nano-fertilisers based on iron oxide (hematite) have the potential to bring significant advantages to agriculture. These nano-fertilisers have been found to enhance plant growth, improve nutrient absorption, and increase photosynthetic efficiency. The application of Fe₂O₃-NPs, specifically the Fe₂O₃-NPs with fulvic acid treatment, significantly improved root nodulation, biomass growth, and chlorophyll content in soybeans (Yang, Alidoust and Wang, 2020). Iron oxide nanoparticles have demonstrated a positive impact on the growth of legume roots, with the extent of growth enhancement being dependent on the dosage. Lower concentrations of these nanoparticles have been found to significantly promote growth, indicating their potential as a fertiliser-like substance (Palchoudhury *et al.*, 2018). The presence of Fe₂O₃-NPs in peanuts resulted in an increase in root biomass and chlorophyll content. Additionally, it caused changes in antioxidant enzyme activities and hormone levels. These findings suggest that Fe₂O₃-NPs can be used as a replacement for conventional iron sources such as EDTA-Fe (Rui *et al.*, 2016). Research conducted on wheat demonstrated that hematite nanoparticles with a size range of 20-40 nm had a notable positive impact on plant biomass, chlorophyll content, and water status. This suggests that these nanoparticles can be effectively used to address iron deficiency in plants without causing any visible harm (Al-Amri *et al.*, 2020). In addition, the application of Fe₂O₃-NPs had a significant impact on the protein, lipid, and fatty acid composition in soybean seeds (Sheykhabaglou, Sedghi and Fathi-Achachlouie, 2018).

Similarly, magnetite nanoparticles are widely discussed in the scientific literature. Studies have shown that nanoforms of iron oxide fertilisers, specifically Fe₃O₄-NPs and its polyethylene glycol-coated variant nFe₃O₄-PEG, have had diverse impacts on plant growth and development. The treatments had minimal impact on germination rates. However, notable changes in seedling radicle development and biomass production were observed, especially when using high concentrations of nanoparticles (Duran *et al.*, 2018; Esper Neto *et al.*, 2021). Maize plants experienced a notable increase in leaf iron content, photosynthesis rate, and biomass when exposed to both Fe and Fe₃O₄-NPs. Importantly, this growth enhancement occurred without causing any oxidative stress, indicating a positive impact on plant development by improving photosynthetic efficiency (P. Li *et al.*, 2020). In contrast, the utilisation of Fe₃O₄-NPs in roselle crops did not enhance, and in certain instances even decreased, the yield and

biomass. This suggests a possible phytotoxicity or lack of effectiveness in specific situations (Bin Shuhaimi, Kanakaraju and Nori, 2019). The results emphasise the intricate nature of nanoparticle interactions with plant systems, emphasising the significance of fine-tuning concentrations and formulations to maximise the advantages of fertilisers containing nanomaterials while minimising potential toxicity.

3.2.1.3 Mineral-based fertilisers

Recent research has investigated the use of hydroxyapatite-based nanomaterials (HA-NPs; chemical composition: inorganic mineral that has a typical apatite lattice structure as $(A_{10}(BO_4)_6C_2)$ where A, B, and C are defined by Ca, PO_4 , and OH) in agriculture, focusing on their improved performance and possible harmful effects. Studies have shown that treatments with HA and HA-NPs can enhance plant growth measurements, such as pod numbers and biomass, without causing significant effects on root nodulation or nitrogen fixation (McKnight *et al.*, 2020). Nevertheless, when present in high concentrations, HA-NPs can potentially cause harm to microalgae by modifying their growth and morphology. However, specific formulations such as hydrothermal HA with ammonium polymethacrylate have been found to have reduced toxicity (Pereira *et al.*, 2017). The utilisation of HA-humic substances nanoparticles leads to a substantial improvement in the release of phosphate ions and crop growth, surpassing the performance of pure HA. This suggests that the functionalization of humic substances plays a crucial role in this enhancement (Yoon *et al.*, 2020). Moreover, the combination of urea and HA in nanohybrids has shown promise in decreasing the amount of urea needed for rice farming while still maintaining or enhancing crop yields (Kottegoda *et al.*, 2017).

Studies on pyrite-based nanomaterials, i.e. iron disulfide mineral, for fertilisers have shown notable progress in enhancing agricultural productivity and sustainability. Moale *et al.* (Moale *et al.*, 2021) demonstrates that surface-nanostructured nanomaterial (SNNM) particle films have a minimal impact on stomatal conductance and photosystem II quantum efficiencies in fruit cultivars. However, specific formulations of these films can effectively lower leaf temperature and alter intercellular CO_2 levels. These modifications result in increased evapotranspiration and net photosynthesis rates, ultimately leading to enhanced water use efficiency and fruit yield, especially in fluctuating weather conditions.

Recent research has shown that zeolite-based nanomaterials (NZNMs) and nano zeolite-composite fertilisers (NZCFs) have promising applications in agriculture. Applying SNNMs and foliar fertilisers has been found to slightly decrease stomatal conductance and photosystem II (PSII) quantum efficiency in stone fruit cultivars, although the statistical significance of these effects is generally not significant in most cases (Moale *et al.*, 2021). Remarkably, these treatments substantially reduced leaf temperature, elevated intercellular CO_2 concentration, and improved net photosynthesis rates, resulting in a significant boost in fruit yield. Furthermore, it has been documented that NZCFs offers a consistent provision of nutrients, leading to a notable enhancement in growth parameters of lettuce through the improvement of soil's physical, chemical, and biological characteristics (M. Z. H. Khan *et al.*, 2021). In addition, nano zeolite (NZ) and zeolite-nitrogen composite (ZNC) materials exhibited lower salt indices than traditional urea. ZNC also demonstrated enhanced water retention abilities, indicating a reduced risk of soil salinity and a favourable slow nutrient release pattern (Lateef *et al.*, 2016).

Recent research emphasises the effectiveness of zincate nano-clay polymer composite (ZNCPC) in enhancing the availability of nutrients in soil and the activities of enzymes. The study (Mandal *et al.*, 2019) demonstrates that ZNCPC treatments greatly improved the levels of pentetic acid extractable zinc and Olsen-P content in different soil types, surpassing the effectiveness of traditional $ZnSO_4 \cdot 7H_2O$ treatments. This improvement was also evident in the heightened levels of dehydrogenase and alkaline phosphatase activity, which serve as indicators of enhanced soil biochemical activity. Furthermore, notable positive correlations among these soil properties indicate a combined effect on soil health. In another study by Zhang *et al.* (S. Zhang *et al.*, 2020) describes the creation of slow-release fertilisers (SRFs) using alginate, bentonite clay, and lignin-clay nanohybrids. Among these, the SRF coated with

poly(acrylic acid)-coated lignin-clay nanohybrid demonstrates the slowest rate of urea release. This formulation not only preserves nutrients but also exhibits a significant ability to retain water, which is especially advantageous in dry environments. The cost-effectiveness of these SRFs in comparison to conventional urea formulations highlights their potential in promoting sustainable agricultural practices.

3.2.1.4 Copper based-fertilisers

The impact of copper oxide nanoparticles (CuO-NPs) on plant physiology and growth varies depending on the concentration and species of the plant. The presence of CuO-NPs in rice had a slight negative effect on germination rates and a positive significant impact on root and shoot growth, biomass, and surface morphology. This resulted in noticeable accumulation of copper, as well as changes in photosynthetic efficiency and pigment content (Da Costa and Sharma, 2016). In contrast, sweet potato plants demonstrated resilience to comparable treatments, as evidenced by the absence of any notable impact on chlorophyll content or photosynthesis. However, there were discernible effects on CO₂ levels and nutrient content in storage roots (Bonilla-Bird *et al.*, 2020). CuO-NPs were primarily found in the vascular and intercellular root spaces of scallions. This led to a significant increase in the accumulation of copper and had an impact on the absorption of both essential and non-essential elements. As a result, the nutritional values and antioxidant responses of scallions were affected (Y. Wang *et al.*, 2020). The growth, antioxidative enzyme activities, and nutrient levels of maize plants were found to be positively influenced within a specific concentration range of CuO-NPs, as indicated by a stimulatory effect. This effect was observed at optimal concentrations of CuO-NPs (Toqeer *et al.*, 2020).

Recent research has demonstrated that copper-based nanoparticles (Cu-NPs) have a dose-dependent impact on plant growth. Low concentrations (between 0.03 mg/mL and 0.06 mg/mL) of Cu-NPs have been found to stimulate growth, while higher concentrations (above 0.43 mg/mL) have been found to hinder it. For example, the presence of biogenic Cu-NPs at low concentrations improved the growth of wheat seedlings and increased their chlorophyll content. However, at higher concentrations, the growth of the seedlings was inhibited (Essa *et al.*, 2021). The application of Cu-NPs to pigeon pea seedlings led to notable improvements in growth, with increased shoot and root lengths, enhanced photosynthetic efficiency, and higher biomass yield. These findings suggest that Cu-NPs have the potential to provide nutritional benefits (Shende *et al.*, 2017). In addition, the application of various forms of copper, bulk Cu or Cu-NPs, to lucerne plants resulted in an increase in the levels of iron and zinc. This had a positive impact on agronomical factors and caused changes in the composition of the microbial community in the potting mix. Nevertheless, elevated concentrations (280 mg/kg) resulted in reduced selenium levels and potential consequences for advantageous microbial communities (Cota-Ruiz *et al.*, 2020).

The presence of Cu-NPs had a negative impact on the growth of cucumber plants. It caused a significant decrease in root length and biomass. This decrease was accompanied by a reduction in essential nutrient elements and changes in metabolite profiles, indicating the plants' response to stress (Zhao *et al.*, 2016). The application of CuO-NPs, bulk CuO, or CuSO₄ on zucchini did not result in any harmful effects on the plant's biomass. These results support previous findings that Cu-NPs does not have an inherent negative impact on the biological parameters of agricultural crops. Nevertheless, the analysis of differential gene expression uncovered specific molecular responses at the nanoscale, indicating nuanced and non-phytotoxic effects of CuO-NPs (Marmiroli *et al.*, 2021). These studies emphasise the subtle effects of Cu-NPs on plant well-being and growth, underscoring the necessity for additional research on their application in agriculture.

3.2.1.5 Silver-based fertilisers

Research has examined the influence of silver nanoparticles (Ag-NPs) on agricultural productivity, revealing diverse outcomes on plant growth and development. Studies have shown that Ag-NPs have the ability to hinder the growth and biomass of wheat, causing phytotoxicity when used in higher doses, at 2000 mg/kg (Yang *et al.*, 2018). However, these nanoparticles can also enter and build up in lettuce

leaves without causing any significant impact on biomass or biochemical markers, even after being washed (Larue *et al.*, 2014). Conversely, it has been observed that lower levels of Ag-NPs can improve the number of leaves, chlorophyll content, and crop yield in onions. This indicates that there may be advantages to using lower doses of Ag-NPs (around 20 mg/kg) compared to higher doses of commercial iron and zinc nanoparticles, which have been shown to be toxic. Moreover, the utilisation of Ag-NPs has demonstrated enhancements in seed germination, resistance against pathogens, chlorophyll content, and crop yield when subjected to stressful conditions. These findings suggest that Ag-NPs have the capacity to augment agricultural productivity (Jaskulski *et al.*, 2022). Furthermore, laboratory and greenhouse conditions have demonstrated that stabilized Ag-NPs have a positive distinct impact on the growth of beetroot roots and stems. However, these nanoparticles do not have a significant effect on the photosynthetic apparatus or the accumulation of heavy elements (Gusev *et al.*, 2016).

Recent research has investigated the harmfulness of Ag-NPs in agricultural settings, emphasising their ability to kill microorganisms and their possible negative effects on the environment. The study by Guilger *et al.* (Guilger *et al.*, 2017) showed that biogenic Ag-NPs successfully suppressed harmful microorganisms and influenced soil bacterial communities that play a role in nitrogen cycling, thereby impacting the balance of microbiota in agricultural soil. In addition, although Ag-NPs demonstrated potential in managing *Sclerotinia sclerotiorum* without affecting the growth of soybean seedlings, it also displayed cytotoxic effects on different cell lines and caused DNA damage. Abdelsalam *et al.* (Abdelsalam *et al.*, 2018) found that exposure of wheat roots to chemically synthesised Ag-NPs resulted in chromosomal abnormalities and cell death. These effects were observed to be dependent on the concentration and duration of exposure, suggesting an interference with the normal cell division process. Interestingly, the germination of the wheat seeds was not affected by the presence of Ag-NPs. The study by Cvjetko *et al.* (Cvjetko *et al.*, 2017) demonstrated that various coatings on Ag-NPs had an impact on their toxicity. Specifically, Ag-NP-cetrimonium bromide significantly hindered root growth and modified the mitotic index. Additionally, it led to an increase in reactive oxygen species and influenced the activities of antioxidant enzymes. These findings highlight the intricate relationship between the antimicrobial advantages of Ag-NPs and their harmful effects on the environment, emphasising the need for cautious evaluation in agricultural uses.

3.2.1.6 Carbon-based fertilisers

Studies on carbon nanotubes as fertilisers indicate that they can improve plant growth when the concentration is properly adjusted. Carboxylic group functionalization enables multi-walled carbon nanotubes (MWCNTs) to separate and spread out in water, which greatly improves the process of mustard seed germination and growth (Subagio, Prihastanti and Ngadiwiyana, 2019). On the other hand, *V. faba L.* demonstrates the most favourable growth when exposed to nitrogen-doped carbon nanotubes with Fe_{1.8}Mn_{1.2}C (NCNTs@NFM). Higher concentrations of NCNTs@NFM (51.2 mg/L) lead to oxidative stress and hormonal imbalances, suggesting toxicity (J. Yang *et al.*, 2021). Similarly, maize exhibits enhanced growth parameters when exposed to MWCNTs.

Recent research on nano-formulated biochar-based fertilisers has shown diverse effects on plant growth, germination, and nutrient absorption. The findings of the study revealed that rice straw biochar nanoparticles (BNPs) have a specific impact on plant growth. They notably promote the growth of rice by significantly increasing the length of its roots and shoots. However, they hinder the growth of reeds while having no effect on tomatoes (K. Zhang *et al.*, 2020). The attachment of nanoparticles to root surfaces, which is affected by the density of epidermal openings, indicates a response specific to plants that needs to be studied more extensively. In contrast, the application of nano-biochar, whether through soil or foliar methods, significantly enhanced growth characteristics, biomass, pigment levels, and biochemical parameters. This improvement exhibited a positive relationship with increasing concentrations, up to 0.5% w/w soil vs. nano-biochar (Khaliq *et al.*, 2023). Furthermore, the carbon nanoparticles loaded with nitrogen (N) and potassium (K) exhibited a notable rate of N and K release,

leading to a substantial improvement in the growth, yield, and nutrient content of common beans. This indicates their effectiveness in delivering nutrients (Salama *et al.*, 2021).

Carbon dots (CDs), also referred as quantum carbon dots, have a typical size of less than 10nm and have shown potential in improving plant growth and nutritional value, as demonstrated in their use with pumpkin seedlings and lettuce. The uptake and toxicity of pumpkin seedlings treated with carbon dots were influenced by the surface charge and concentration of cyclodextrins on the dots, resulting in reduced biomass and increased lipid peroxidation (Qian *et al.*, 2018). This emphasises the significance of surface modification in reducing the harmful effects on plants (phytotoxicity). In contrast, when nitrogen-doped carbon dots (N-CDs) were applied to lettuce leaves, it resulted in a significant increase in growth and improved nutrient content. This effect was dependent on the concentration of N-CDs, up to 200 mg/L, which led to an increase in biomass accumulation and enhanced photosynthetic efficiency. Importantly, there were no negative effects on food safety. These findings indicate that N-CDs have the potential to be a novel fertiliser solution, enhancing sustainable agriculture by enhancing crop productivity and quality through the precise design and application of nanomaterials (Tan *et al.*, 2023).

3.2.1.7 Silicon-based fertilisers

Multiple studies have shown that silica nanoparticles (SiO₂-NPs) have intricate impacts on both the growth and physiology of plants. The presence of SiO₂-NPS at low concentrations, such as 50-100 mg/L, enhances the root weight and water absorption in wheat without causing any significant changes in shoot weight. This indicates that these levels of SiO₂-NPs improve the plant's ability to withstand stress. Conversely, elevated concentrations, up to 800 mg/L, result in a reduction in shoot weight, an increase in lipid peroxidation, and an elevation in catalase activity, suggesting the possibility of toxicity and stress (Karimi and Mohsenzadeh, 2016). In maize, the addition of SiO₂-NPs has been found to enhance the chlorophyll content and biochemical components, including proteins and phenols. This leads to improved stress tolerance and metabolic balance. However, it is important to note that high concentrations (500 µg/mL) of SiO₂-NPs may have toxic effects on osteoblast cell lines (R. Suriyaprabha *et al.*, 2014). In the plant species *A. hypogaea*, the addition of SiO₂-NPs has a significant positive effect on growth metrics. This indicates that it enhances physical barriers and stress resistance. However, the benefits start to decrease when the concentration is above 400 ppm (Prasad *et al.*, 2023). The application of SiO₂-NPs to wheat results in enhanced growth parameters, increased chlorophyll content, and heightened metabolic activity, suggesting a significant improvement in both growth and physiological responses (Y. Li *et al.*, 2023).

The incorporation of silicon nanoparticles (Si-NPs) and sodium silicate increases the concentration of silicon in plants, leading to a significant impact on the absorption of silicon by both roots and shoots. Notably, nanoparticles demonstrate a similar level of effectiveness as sodium silicate. The heightened deposition of lignin in the cell walls of xylem and the heightened activity of enzymes, such as phenylalanine ammonia-lyase, suggest an enhancement in the resilience of plants and their metabolic processes. Si-NPs and sodium silicate have comparable effects both on the activities of antioxidant enzymes and the expression of genes, highlighting their contribution to stress tolerance and the promotion of growth. Nevertheless, Si-NPs possess distinctive physicochemical characteristics that enhance their bioavailability and efficacy in terms of plant absorption and stress reduction, surpassing those of bulk silicon. When applied through seed priming, Si-NPs have demonstrated encouraging improvements in growth and productivity across different plant species, thus having potential advantages. Their toxicity is primarily caused by pH alterations in growth media. Therefore, it is crucial to carefully evaluate particle size, concentration, and application methods to optimise agricultural benefits while minimising negative consequences (Nazaralian *et al.*, 2017; Bhat *et al.*, 2021).

3.2.1.8 Titanium-based fertilisers

The utilisation of titanium dioxide nanoparticles (TiO₂-NPs) in agricultural activities, as supported by several studies (Moll *et al.*, 2016; Missaoui *et al.*, 2017; Shenavaie Zare *et al.*, 2022; Pérez-Velasco *et al.*,

2023), exhibits substantial effects on the growth of plants, absorption of nutrients, and antioxidant reactions. Specific concentrations and formulations of TiO₂-NPs result in improved fruit quality, higher levels of carotenoids and anthocyanins, and enhanced growth metrics. Nevertheless, higher concentrations could potentially diminish growth rates and impact the movement of nutrients. The addition of TiO₂-NPs to salicylic acid or methyl jasmonate enhances positive results, such as increased antioxidant activity and production of secondary metabolites.

In one study, results indicate that TiO₂-NPs have minimal impact on the absorption, movement, and build-up of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in pumpkin seedlings, even at concentrations that are relevant to the environment (above 5 mg/L). No changes were observed in chlorophyll production, anthocyanin content, and seedling mass following exposure. The lack of competition in the uptake of PFOA/PFOS and TiO₂-NPs indicates that they follow different transport pathways, with aquaporins playing a vital role in the uptake of TiO₂-NPs. The minimal adsorption of PFOA and PFOS on TiO₂-NPs emphasises that electrostatic repulsion may be a potential mechanism (Xu *et al.*, 2019).

3.2.1.9 Selenium-based fertilisers

Recent research has provided a comprehensive understanding of the diverse functions of selenium nanoparticles (Se-NPs) in agriculture, emphasising their ability to improve plant growth and increase resistance to stress. The study by Sonali *et al.* (Sonali *et al.*, 2023) showed that biogenic Se-NPs have a notable impact on scavenging free radicals, improving soil moisture and nutrient content. As a result, they enhance various agronomic characteristics of plants, but do not possess antibacterial properties against common pathogens. A separate investigation by Alsherif *et al.* (Alsherif *et al.*, 2023) discovered that Se-NPs, particularly when exposed to higher levels of carbon dioxide, significantly enhanced the levels of important photosynthetic pigments, antioxidant capabilities, and vital nutrients in plants. This finding further supports the potential of Se-NPs in enhancing plant productivity. Furthermore, the study published by Gudkov *et al.* (Gudkov *et al.*, 2020) demonstrated that Se-NPs are effective in reducing the impact of high-temperature stress. The research also identified an ideal concentration of 10 µg/kg of Se-NPs that can provide long-lasting benefits and alleviate stress in different crops. These findings highlight the important equilibrium between improved functionality and the requirement for accurate dosage and distribution to utilise the advantages of Se-NPs in agriculture while reducing environmental risks.

Chitosan oligosaccharide coated Se-NPs improved nutritional parameters and element concentrations within the ideal range of 2.36–9.43 mg/L for soybean sprouts. The selenium enrichment has a significant effect on the distribution of selenium that is bound to proteins. Nevertheless, elevated selenium concentrations (above 9.43 mg/L of Se-NPs) stimulate the production of reactive oxygen species, which hinders the synthesis of proteins and modifies the binding of selenium to large molecules. This emphasises the importance of maintaining a careful equilibrium in the use of nanoforms for agricultural improvements (Xin Xu *et al.*, 2023).

3.2.1.10 Chitosan-based fertilisers

The utilisation of thiamine-chitosan nanoparticles (TCNPs) and chitosan nanoparticles (CSNPs) in conjunction with arbuscular mycorrhizal fungi (AMF) has exhibited substantial enhancements in plant germination, growth, and defence mechanisms. The application of TCNP treatment led to an almost 90% increase in germination rate, a tenfold rise in indole-3-acetic acid concentration, improved seedling strength, and enhanced growth characteristics including root and shoot length. Furthermore, it effectively promoted nodulation in chickpea seedlings (Muthukrishnan, Murugan and Selvaraj, 2019). Although TCNP does not possess direct antifungal properties, it provides resistance against *Fusarium oxysporum* complex by promoting the accumulation of defence enzymes. This suggests that TCNP functions as a plant growth regulator and activator of defence mechanisms. In the same manner, the use of CSNPs, especially when combined with AMF, resulted in a significant increase in the content of indole-3-acetic acid, photosynthetic rate, and sugar levels in wheat. Additionally, it enhanced the levels of

antioxidants and phenolic compounds without causing a significant change in the weight of the wheat, whether fresh or dry (Saleh *et al.*, 2023).

Chitosan with poly(methacrylic acid) (CS-PMAA) nano-fertilisers have shown significant agricultural advantages as well as possible toxicity, which depends on the dosage used. Studies have shown that when using an ideal concentration, there are noticeable improvements in plant growth, root biomass, and nutrient absorption. Additionally, there are benefits in managing soil water and nutrient cycling, particularly for potassium and phosphorus (Kubavat *et al.*, 2020). In contrast, elevated concentrations demonstrate toxicity that is dependent on the dosage, resulting in inhibited growth of roots, increased death of seedlings, and DNA damage, despite some promotion of growth at lower doses (Khalifa and Hasaneen, 2018). The contrast between these two aspects highlights the significance of optimising the dosage of CS-PMAA nano-fertilisers in order to ensure their safe and efficient application in agriculture.

The study by Wang *et al.* (C. Wang *et al.*, 2020) found that the release of urea from halloysite nanotubes with chitosan (Hal@CS) was slower compared to raw halloysite nanotubes, but it was accelerated in solutions containing glutathione. Hal@CS improved seed germination and promoted root growth without causing any immediate harm. The infiltration of nanoparticles into root cells indicates that Hal@CS has the potential to function as a controlled-release system for delivering fertilisers, thereby enhancing crop absorption and promoting growth.

3.2.1.11 Polymer-based fertilisers

Research on nano-fertilisers made from poly(vinyl alcohol) has shown notable progress in the controlled release of nutrients and their interaction with the environment. Sharma *et al.* (Sharma, Singh and Dutta, 2021) explored a polymer-clay composite that undergoes a transition into a hydrogel when exposed to soil. This transformation allows for a controlled and gradual release of nutrients, which is essential for promoting sustainable agriculture. This process, facilitated by the absorption of water, exhibits a gradual yet progressive rate of release, particularly in environments abundant in organic compounds, while also demonstrating a significant ability to undergo biodegradation. An article by Liu *et al.* (S. Liu *et al.*, 2021) emphasises the gradual release of nitrogen, phosphorus and potassium fertilisers through Fickian diffusion in polymer-based hydrogels, which complies with European regulations for slow-release fertilisers and minimises environmental harm. Kumar *et al.* (Kumar, Ashfaq and Verma, 2018) demonstrates the beneficial impact of nano-fertilisers containing two metals on plant growth. These nano-fertilisers greatly improve germination, shoot length, and the availability of micronutrients, all without causing harm to the plants.

Slow-release fertilisers (SRFs) based on poly(acrylic acid-co-acrylamide) exhibit improved agricultural efficiency by releasing nutrients in a controlled manner, thereby reducing nutrient leaching and minimising environmental impact. The H100 treatment, which utilised nanocomposite hydrogels, demonstrated the most gradual release rates for vital nutrients, leading to increased root dry weight, fruit dry mass, and enhanced nutrient retention in the substrate (Motamedi, Safari and Salimi, 2023). This formulation not only reduced nutrient waste, but also enhanced the levels of essential nutrients and antioxidants in tomatoes, demonstrating its superior agronomic performance and environmental advantages. In addition, modifying the composition of the polymeric coating and incorporating nano-clay nanoparticles have been found to enhance nutrient release behaviours, increase soil water holding capacity, and greatly reduce nitrate leaching (Salimi *et al.*, 2020).

The research (S. Zhang *et al.*, 2021; Liu *et al.*, 2022) showcases the progress made in developing controlled-release fertilisers using polyurethane-based nano-formulation. The studies demonstrate that the release rates of phosphorus and nitrogen from these fertilisers can be precisely controlled by manipulating factors such as the duration of water immersion, the thickness of the film, and the incorporation of carbon nanotubes hydroxyl (CNT-OH) and coating percentages. More precisely, the addition of CNT-OH increases the release of phosphorous up to a certain point. However, beyond this

point, it becomes detrimental because it reduces the cross-linking of the membrane and increases the surface energy. Organosilicon- polydimethylsiloxane and nano-silica modifications in fertilisers create a superhydrophobic effect similar to the lotus leaf, which prevents water intrusion. This effect, known as super-hydrophobicity, significantly extends the release of nutrients by forming a "air shield". The results emphasise the possibility of using precisely designed nano-enhanced controlled-release fertilisers in agriculture. These fertilisers have customised nutrient release patterns that can enhance efficiency and minimise harm to the environment.

3.2.1.12 Urea-based fertilisers

The studies conducted on various documents clarify the advantageous effects of nano-formulated urea and other nutrient-enhanced formulations on the growth, productivity, and soil quality of crops. The utilisation of fertiliser N, specifically when incorporating urea nanoparticles, significantly enhanced the growth of maize plants, grain production, and absorption of nutrients, yielding results similar to those obtained from traditional urea applications. Significantly, this method also alleviated the occurrence of slight leaf damage that was noticed with traditional urea sprays, indicating a possible decrease in plant toxicity. Similarly, rice experienced positive effects from urea nanoparticles (TNU), as there was no notable variation in plant height, but there was an increase in grain yield and nitrogen uptake. The results emphasise the effectiveness of nano-formulations in improving crop productivity while potentially decreasing the amount of fertilisers needed. Incorporating biochar into fertiliser granules, as outlined in a separate investigation, enhanced shell porosity and facilitated moisture infiltration. This could potentially lower soil acidity, minimise nutrient loss, and decrease the overall reliance on fertilisers and irrigation. This alteration proposes a promising approach to improve soil health and increase crop efficiency. Applying nitrogenous fertilisers to pomegranates via the leaves resulted in an increase in the concentration of nitrogen in the leaves, as well as an improvement in fruit yield and quality. Nevertheless, elevated levels of urea resulted in leaf damage, emphasising the significance of fine-tuning the dosage in foliar fertilisation approaches. The growth and yield parameters of sorghum were significantly enhanced when nano urea was applied, either through soil or foliar methods. The findings suggest that nano-formulations can efficiently provide nutrients, resulting in improved crop productivity. These studies collectively demonstrate the potential of using nano-formulated and modified nutrient carriers in agriculture to increase crop yield, improve nutrient efficiency, and potentially support sustainable farming practices (Davaranah *et al.*, 2017; Bolshanina *et al.*, 2023; Singh *et al.*, 2023).

3.2.1.13 Amino acid-based fertilisers

Recent research has shown that using nanoform fertilisers made from amino acids can increase agricultural productivity and may have harmful effects on cells. According to Abdelsalam *et al.* (Abdelsalam *et al.*, 2019), wheat that was treated with a foliar application of 75% nitrogen, phosphorous and potassium (NPK) nanoparticles showed notable enhancements in growth, such as increased spike length, grain yield, and nutrient content. However, it also demonstrated an increase in cellular abnormalities and a higher mitotic index, indicating possible genotoxicity. Pooja *et al.* (Pooja *et al.*, 2022) examined the favourable effects of applying nano-N fertiliser at a dosage of 50% of the recommended amount on plant growth, yield characteristics, and quality parameters. This treatment resulted in the highest benefit-cost ratio. This highlights the increased effectiveness of nanoform fertilisers in agricultural applications.

3.2.1.14 Boron-based fertilisers

Studies have shown that the use of nanoform fertilisers leads to improved plant growth and productivity. Notably, the application of potassium and boron nanoparticles has resulted in substantial enhancements in chlorophyll content, plant height, and grain yield. According to a study by Noaema *et al.* (Noaema, Leiby and Alhasany, 2020), applying these nanoparticles to the leaves not only boosts the amount of chlorophyll, resulting in better photosynthesis, but also amplifies the quantity of spikes per square metre, grains per spike, and overall grain and biological yield. Kandil *et al.* (Kandil *et al.*, 2020)

also shows that the use of NPK nanoparticles and fulvic acid has a significant positive effect on root parameters, shoot and root yield, sugar content, and purity in sugar beetroot. This study emphasises the importance of boron in promoting root development and enhancing sugar yield. The results emphasise the capacity of nanoform fertilisers in agriculture to enhance efficacy.

The application of various sources of boron, including nanoforms, to sunflower plants did not have a significant effect on the weight of the plants in different types of soil. However, it did significantly increase the concentration of boron in the plant tissues, especially in young leaves. This suggests that the plants were able to absorb nutrients more efficiently. Significantly, the application of nano-boron and other boron sources caused changes in calcium levels in the young leaves of plants cultivated in alkaline soil, indicating a complex relationship between boron application and calcium absorption. In contrast, a distinct investigation demonstrated that nano-fertiliser greatly increased the amount of above-ground and below-ground plant material in lettuce and zucchini, surpassing both untreated samples and conventional commercial fertilisers. This indicates the potential of nano-fertilisers in enhancing plant growth and improving nutrient delivery efficiency. Nevertheless, an overabundance of boron, particularly when administered through nutrient solutions and foliar applications, resulted in a decrease in growth and indications of toxicity. This highlights the significance of carefully controlling the dosage when utilising nano-formulated fertilisers to prevent any harmful consequences. The results emphasise the intricate relationship between the use of nano-formulated nutrients, the growth of plants, and the possibility of nutrient-specific movement and toxicity problems. These factors are crucial for improving agricultural practices and fertiliser compositions (Meier *et al.*, 2020; Aydogan, Taskin and Gunes, 2023).

3.2.1.15 Calcium-based fertilisers

Calcium phosphate-based nano-fertilisers, such as amorphous calcium phosphate nanoparticles doped with potassium, nitrate, and urea (nano-U-NPK), provide novel methods to increase agricultural productivity while using less nitrogen. The study by Ramírez-Rodríguez *et al.* (Ramírez-Rodríguez *et al.*, 2020) demonstrates that nano-U-NPK effectively slows down the process of urea leaching, while also maintaining a high level of compatibility with living cells. This confirms that nano-U-NPK is a reliable method for delivering nitrogen in a controlled manner, without causing any negative effects. Similar growth and yield to control plants, even though they had less nitrogen. This indicates that nano-fertilisers can help plants absorb nutrients more efficiently. In addition, the article by Carmona *et al.* (Carmona *et al.*, 2021) presents a method for modifying a material after synthesis, which greatly improves the efficiency of nitrogen content and showcases its capability for gradual and controlled release of nutrients. The results emphasise the ecological and agricultural advantages of employing nano-fertilisers, which aid in promoting sustainable farming methods by reducing nutrient wastage and enhancing plant nutrient absorption.

3.2.1.16 Magnesium-based fertilisers

Nano-fertilisers containing magnesium oxide (MgO-NPs) have a notable impact on plant growth and physiological characteristics, without causing any toxic effects. Research findings demonstrate that MgO-NPs is highly effective in enhancing the levels of chlorophyll, boll weight, seed cotton yield, and essential nutrient concentrations in cotton plants (Kanjana, 2020). Likewise, tobacco seedlings that were treated with MgO-NPs showed an increase in chlorophyll levels and improved activities of antioxidant enzymes, without causing any harmful effects on the structure or appearance of the plants. Significantly, the treatment with MgO-NPs results in a substantial enhancement in the accumulation of magnesium in plant tissues, thereby promoting better distribution of nutrients (Cai *et al.*, 2018).

3.2.1.17 Manganese-based fertilisers

The application of nanoforms of manganese (Mn) as a fertiliser has variable effects on wheat growth and nutrient absorption but does not have a significant impact on dry matter yield, tiller number, or plant height. Mn-NPs reduces the accumulation of Mn in shoots and modifies the distribution of nitrogen

and phosphorous, resulting in a decrease in nitrogen content in above-ground parts and a negative impact on the uptake of phosphorous and potassium. In contrast, the application of Mn-NPs to soil and foliage has varying effects on the movement of nutrients, specifically resulting in a decrease in potassium concentration in the shoots when using nano Mn. Mn-NPs augment nitrogen metabolism in plants by elevating the activities of nitrate reductase and nitrite reductase, thereby indicating enhanced nitrogen assimilation. During toxicity studies, Mn-NPs has been found to be biocompatible at recommended doses in mice, showing minimal to no histological abnormalities. However, it is important to note that high concentrations of Mn-NPs may have a potential impact on mitochondrial function. The results emphasise the subtle influence of Mn-NPs on plant growth and nutrient dynamics, which has implications for agricultural practices and the design of nano-formulations (Pradhan *et al.*, 2014; Dimkpa *et al.*, 2018).

3.2.1.18 Cerium-based fertilisers

The presence of cerium oxide nanoparticles (CeO₂-NPs) greatly increases the amount of roots in wheat plants, regardless of whether the soil has low or high nitrogen levels. The effects are particularly noticeable in low nitrogen conditions, as indicated by strong statistical analyses. While the shoot biomass and grain yield were not impacted by low nitrogen levels, changes in the elemental composition of the grain were observed. Specifically, there was a significant increase in iron concentrations and a decrease in phosphorus, potassium, calcium, and manganese at low nitrogen. The results indicate that CeO₂-NPs enhance root growth without any negative impact on the overall grain yield, thus emphasising their potential in facilitating plant development in conditions where nutrients are limited (Rico *et al.*, 2020).

3.2.1.19 Gold-based fertilisers

The study by Avellan *et al.* (Avellan *et al.*, 2019) reveals that the adhesion and uptake of gold nanoparticles (Au-NPs) on wheat leaves differ depending on their size and coating. Polyvinylpyrrolidone-Au-NPs exhibit a higher level of leaf association and uptake compared to citrate-coated Au-NPs. Gold nanoparticles have distinct effects on the movement of substances across cell membranes and the overall well-being of plants, affecting the process of photosynthesis and the amount of plant matter produced.

3.2.2 Fungicides

Fungicides are substances designed to kill or inhibit fungi and their spores, crucial for controlling plant diseases. Examples of targeted fungi include *Botrytis cinerea*, causing grey mould, and *Puccinia spp.*, responsible for rust diseases, as well as spores like those from *Phytophthora infestans*, causing late blight.

3.2.2.1 Aluminium-based fungicides

Recent studies on aluminium-based nanomaterials, like the nano-conjugated Boscalid@ZIF-67 described by Zhang *et al.*, revealed significantly improved antifungal efficacy against *B. cinerea* if compared to traditional available fungicide agents, while also maintaining a much lower bio-toxicity (Zhang *et al.*, 2022). Moreover, composite nanomaterials such as Ni_{0.5}Al_{0.5}Fe₂O₄-NPs demonstrated exceptional antifungal activity against *F. oxysporum* in ginger, both in vitro and in vivo, completely eliminating the disease (Sharma *et al.*, 2022). Thus, aluminium based nano-fungicides showed a dual effect on plants, both beneficial and toxic, meaning that accurate dosing and application procedures are necessary for managing agricultural diseases effectively.

3.2.2.2 Bioavailable molecule-based fungicides

Novel bioavailable nanomaterials are changing the landscape of fungicidal applications by improving safety and efficacy. Azoxystrobin-layered double hydroxide (LDH) nanocomposites provided enhanced dispersion and delayed release of the fungicide agent in aqueous settings, allowing for targeted distribution with up to 70% foliar adhesion (Zhi *et al.*, 2022). In a study performed by Sharma *et al.*, the

combination of clove and lemongrass oil (CO-LGO) produced synergistic antifungal effect which was significantly enhanced by nano-emulsification. In several application modes, this formulation showed strong efficacy against *F. oxysporum*, considerably reducing the disease (Sharma *et al.*, 2018). LDH nanosheets appears to be nontoxic, biodegradable in the environment, and to have strong adhesion that makes them resistant to be washed away (Zhi *et al.*, 2022). Moreover, it has been demonstrated that the clove/lemongrass nano-emulsion used as fungicide was biocompatible and had no antiproliferative effect on human HEK 293T cells, even at high doses (Sharma *et al.*, 2018), suggesting their safety in situations involving non-target exposure.

3.2.2.3 Calcium-based fungicides

In a research published by Yunhao *et al.*, calcium-based nano-fungicides, particularly prochloraz-mesoporous organosilica-calcium carbonate (PRO-MON-CaC) nano-formulation, exhibited a concentration-dependent fungicidal activity similar to the control, delivering sustained protection against fungal challenges. PRO-MON-CaC outperformed conventional formulations in terms of long-term efficacy due to its unique MON-CaC carrier, which allowed for extended release and prevented the degradation of the active ingredient (Gao *et al.*, 2020). In the same study, in vivo toxicity assessments using zebrafish revealed that PRO-MON-CaC was less hazardous than PRO technical, with not significant plant safety concerns at the applied doses.

3.2.2.4 Carbon-based fungicides

Carbon-based fungicides in nanoforms have emerged as a promising alternative to conventional ones, offering superior efficacy, targeted delivery, and reduced environmental impact. According to a study published by Fatemi *et al.*, methyl jasmonate and multi-walled carbon nanotubes dramatically increased the amount of rosmarinic acid in *S. khuzistanica* cultures, simultaneously enhancing antioxidant activities and suppressing the growth of *F. solani*. (Fatemi *et al.*, 2020). Additional investigation demonstrated an increased and size-dependent antifungal activity against *F. oxysporum* of reduced graphene oxide-copper oxide NPs (El-Abeid *et al.*, 2020). Furthermore, the graphene oxide-pyraclostrobin nanocomposite exhibited enhanced antifungal activity and outstanding release control when compared to its constituents alone (Peng *et al.*, 2022). Comparable fungicidal activity was shown by tebuconazole@porous carbon nano-particles@chitosan nano-pesticides in comparison to their active ingredient counterparts, suggesting effective pesticide release and the potential to lower total pesticide consumption (Dong *et al.*, 2021). Studies on carbon dots (CDs) loaded with flumorph showed increased antifungal action, indicating that CDs could be useful as pesticide transporters (Zhao *et al.*, 2021). Further research on carbon quantum dots as carriers for insoluble pesticides revealed that the solubility, absorption and translocation of the active chemicals within plants were size-dependent (Xu *et al.*, 2021). Importantly, safety profile found concerning the toxicity of carbon-based nano-fungicides. For example, in the absence of laser irradiation, CDs demonstrated low in vitro cytotoxicity to both malignant and normal cells (Zhao *et al.*, 2021).

3.2.2.5 Cerium-based fungicides

Cerium oxide nanoparticles (CeO₂-NPs) have demonstrated to enhance the plant resistance against fungal diseases in tomato plants, improving chlorophyll content and photosynthetic capacity (Adisa *et al.*, 2018, 2020). Furthermore, biosynthesized CeO₂-NPs considerably increased the morphological characteristics, defence enzyme activities, and antioxidant responses of wheat crops, offering strong defence against *P. striiformis* (Shahbaz *et al.*, 2022). Together, these investigations showed how CeO₂-NPs can improve plant health and yield while reducing the effects of fungus-related diseases.

3.2.2.6 Chitosan-based fungicides

Several recent studies on chitosan nanomaterials used as fungicides have shown promising results. Chitosan nanoparticles (CNPs) revealed to have a dual effect of fungal disease control and growth promotion, as demonstrated in rice and chickpea plants (Sathiyabama and Parthasarathy, 2016; Sathiyabama and Muthukumar, 2020). Novel chitosan formulations, exhibited superior and longer-

lasting anti-fungal properties compared to conventional chitosan solutions (Popova *et al.*, 2023). Synthesised tripolyphosphate-chitosan (Maluin, Hussein, Yusof, Fakurazi, Idris, *et al.*, 2019) or chitosan-hexaconazole-dazomet nano-particles (Maluin, Hussein, Yusof, Fakurazi, Abu Seman, *et al.*, 2019) exhibited strong size-dependent antifungal activity against *G. boninense*, with notable inhibitory effects seen even at low doses. In another study it has been demonstrated the long-term antifungal activity of chitosan@protocatechuic acid nanoparticles against *P. oryzae*, proving the benefits of combining natural compounds with CNPs (Pham *et al.*, 2019). CNPs have been also used as nanocarriers to encapsulate active ingredient, as reported in the study performed by Nadendla *et al.* (Nadendla *et al.*, 2018). When compared to non-encapsulated variants, tomato leaves treated with HarpinPss encapsulated in CNPs had a greater and quicker rate of *R. solani* cell death.

The development of CNPs antifungal agents demonstrated encouraging safety and biocompatibility characteristics. Notably, porous carbon nano-particles@chitosan nanocarriers preserved human hepatic cells ability to proliferate and showed an enhanced safety margin in mice models (Dong *et al.*, 2021). A remarkable study has also demonstrated a controlled release of the active ingredient from the nanoparticles and verified that there were no harmful fungicide residues in palm oil, guaranteeing consumer safety (Maluin *et al.*, 2020). However, there is still a need for cautious dosage control because of concerns about the possible negative effects of high concentrations of certain CNPs (El-Naggar *et al.*, 2022). Positively, rhizobacteria like *B. licheniformis* did not exhibit any inhibitory effects at the investigated concentrations of CNPs, indicating a safe application range (Panichikkal, Puthiyattil, *et al.*, 2021). Nevertheless, caution is always necessary because high concentrations of some bimetallic blends and chitosan nanocomposites demonstrated to have genotoxic effects (Abd-Elsalam *et al.*, 2017).

3.2.2.7 Cobalt-based fungicides

The effectiveness of cobalt-based nanoparticles in agricultural applications has been shown by recent investigations. In particular, it was discovered that cobalt oxide nanoparticles (Co_3O_4 -NPs) exhibited a strong potential for the treatment of *F. wilt* disease (El-Sayed *et al.*, 2023). Furthermore, cobalt ferrite nanoparticles (CoFe_2O_4 -NPs) showed strong antifungal action, effectively controlling *F. wilt* and inhibiting *C. gloeosporioides* mycelial growth by up to 81.39% (Sharma *et al.*, 2017).

3.2.2.8 Copper-based fungicides

Copper oxide nanoparticles (CuO -NPs) demonstrated remarkable efficacy as nanosized fungicides. As an example, they showed to significantly inhibit the mycelial growth of various fungi such as *Fusarium* and *B. cinerea*, crucial pathogens affecting tomatoes and roses, respectively (Elmer and White, 2016; Hao *et al.*, 2017, 2019). CuO -NPs not only prevented disease but also promoted healthier plant growth, evidenced by increased root copper levels in *P. vulgaris* plants (El-Sayed *et al.*, 2023). It was also shown that Cu -NPs have antifungal action. Research has demonstrated its efficacy against several fungal diseases, including *C. lunata* and *P. destructiva* (Kanhed *et al.*, 2014). Moreover, studies of Cu and CuO nanoparticles in contact with fungal hyphae demonstrated a substantial immobilisation by the fungal structures, indicating a potentially robust interaction mechanism influencing the antifungal activity of the nanoparticles (Kovačec *et al.*, 2017). Furthermore, a study on copper phosphate ($\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$) nanosheets demonstrated their capacity to suppress the growth of disease and reduce fungal biomass, at concentrations noticeably lower than those needed for CuO -NPs (Borgatta *et al.*, 2018). CuO -NPs synthesised with *Azadirachta indica* (neem) extract showed strong antifungal action against pathogens that predominate in apple orchards (H. Ahmad *et al.*, 2020). It was proven that the combination of CuO -NPs and neem extract synergistically inhibited fungal growth more effectively than each component acting alone. According to Lopez-Prieto *et al.* research, the combination of the fungicide copper-oxychloride with an extract of corn steep liquor biosurfactant greatly suppressed the growth of *B. cinerea* by enhancing copper-oxychloride penetration through pathogen cells, suggesting a potential strategy to improve fungicide delivery (López-Prieto *et al.*, 2023). In another research study, a formulation including Cu -NPs and essential oils outperformed traditional fungicides like mancozeb and carbendazim against maize pathogens (Dorjee *et al.*, 2023). Notably, myco-fabricated Cu -NPs showed

significant inhibition against a variety of plant pathogenic fungi, including *A. niger*, *F. oxysporum*, and *A. alternata*, with *A. niger* being the most sensitive (Shende *et al.*, 2021).

Recent investigations into the toxicity of copper-based nano-fungicides elucidated their impact. Cu₂O-NPs exhibited high acute toxicity in zebrafish, suggesting limited suitability for use as fungicides if compared with other copper-based NPs (Yuan *et al.*, 2023). CuO-NP@alginate nanogel@polylysine demonstrated to be less hazardous for agricultural use than CuCaSO₄, with the toxicity being attributed to copper ion concentration-related effects (Zhu *et al.*, 2022). The availability of free Cu²⁺ ions in the soil solution, correlated with the toxicity of copper, regardless of its nanoform, suggested that the effect is non-nanospecific (Kah *et al.*, 2022). Cu/Cu₂O-NPs treated plants experienced transient phytotoxic symptoms, indicating a thin boundary between phytotoxicity and efficacy (Giannousi, Avramidis and Dendrinou-Samara, 2013). According to *in vitro* research, lung cells were more sensitive to copper nanoparticles than kidney cells were, thus precautions must be taken while applying them (Sadek *et al.*, 2022).

3.2.2.9 Gold-based nanoparticles

Recent advancements in nanotechnology led to the development of gold-based fungicides showcasing potent antifungal activities. Gold-chitosan NPs exhibited a dose-dependent antifungal efficacy against *F. oxysporum* strains (Lipša *et al.*, 2020). Further exploration showed that gold nanoparticles (Au-NPs), particularly those made from endophytic fungal biomass, prevented *R. solani* sclerotia formation by up to 93% at a concentration of 80 µg/mL (Soltani Nejad *et al.*, 2022). Additionally, biosynthesized nanoparticles from *T. atroviride* significantly inhibited the growth of *P. theae* (Ponmurugan, 2017). In terms of structural influence on antifungal efficiency, branched gold nanomaterials had a significant inhibitory impact against *F. solani*, whereas spherical Au-NPs did not exhibit similar effects (Osonga, Eshun and Sadik, 2022).

Due to their great biocompatibility and intrinsic low cytotoxicity, Au-NPs are being used more and more in agricultural contexts, which makes them a good choice for scaffold applications (Osonga, Eshun and Sadik, 2022).

3.2.2.10 Iron-based nanoparticles

Researches on iron-based nanomaterials demonstrated that iron oxide nanoparticles (Fe₂O₃-NPs) and iron sulphide nanoparticles (FeS-NPs) significantly enhanced antifungal activity and biochemical responses. In tomatoes and cucumbers, Fe₂O₃-NPs at varying concentrations effectively reduced disease severity caused by *Fusarium spp.*, improved photosynthetic pigment content, and increased levels of soluble carbohydrates, proteins, and phenolic compounds (Elbasuney *et al.*, 2022; El-Batal *et al.*, 2023). These improvements indicated a strengthened plant defence mechanism and enhanced systemic resistance. Similarly, FeS-NPs showed superior efficacy against *F. verticillioides*, offering a potent and eco-friendly alternative to traditional fungicides (Ahuja, Sidhu and Bala, 2019). Furthermore, magnetite (Fe₃O₄-NPs) not only improved seed germination and seedling growth across various crops but also exhibited a broad spectrum of antifungal activities, indicating their potential as effective antimicrobial agents (Win *et al.*, 2021). The development of prochloraz nano-capsules incorporating Fe₃O₄-NPs enhanced the delivery and stability of fungicides, demonstrating increased adhesion and photostability (Xue *et al.*, 2020). In another published research, Ni_{0.5}Al_{0.5}Fe₂O₄-NPs exhibited excellent antifungal activity against *F. oxysporum* in ginger (Sharma *et al.*, 2022). Moreover, chitosan-Fe₂O₃-NPs, with increased chitosan concentration, outperformed conventional fungicides in controlling *R. oryzae* in strawberries, demonstrating their superior disease suppression capabilities (Saqib *et al.*, 2019).

However, the use of iron-based fungicide nanomaterials in agriculture presented a dual aspect of beneficial and toxic effects on plants and animals, emphasizing the critical importance of understanding and managing their application (Sharma *et al.*, 2022). Even while these nanomaterials effectively prevented plant diseases, their interactions with biological systems showed a complicated toxicity

profile. By disrupting cell membrane permeability and fostering non-specific interactions, which result in increased intracellular accumulation, positive surface charges on iron oxide nanoparticles demonstrated genotoxic and cytotoxic consequences (Ashraf, Anjum, *et al.*, 2022). On the other hand, it was observed that some iron-based nanoparticles are not harmful to humans or phytotoxic to plants, suggesting that, when used appropriately, they could be safely used in agriculture (Ashraf, Batool, *et al.*, 2022; S. Khan *et al.*, 2022). In a research study, long-term exposure to elevated concentrations of Fe₃O₄/hydroxyapatite nanoparticles caused mild to severe histopathological alterations in the liver and kidney tissues, as well as significant haematological changes in rats, including increases in haemoglobin levels and red blood cell counts. These results pointed to possible toxicity hazards with longer exposure times or at larger doses (El-Ganainy *et al.*, 2022). Furthermore, compared to other metal nanoparticles, iron nanoparticles was less toxic to aquatic life, suggesting that iron-based nanomaterials should be used for agricultural purposes rather than others, like silver nanoparticles (Tesser *et al.*, 2022).

3.2.2.11 Magnesium-based fungicides

The fungicide activity of magnesium-based nanoparticles has been shortly reported in the literature. For example, aqueous magnesium oxide nanoparticles (MgO-NPs) exhibited potent antifungal activity, with fungitoxicity being concentration-dependent against *F. fujikuroi* (222 µg/mL), *B. oryzae* (242 µg/mL), and *F. verticillioides* (249 µg/mL) (Sidhu *et al.*, 2020). In a further investigation MgO-NPs revealed complete fungal growth inhibition at 15.36 µg/mL for *F. oxysporum* and biofilm formation prevention at 1.92 µg/mL, underscoring the nanoform capability to significantly obstruct fungal proliferation in a dose-dependent manner (Abdel-Aziz, Emam and Elsherbiny, 2020; Chen *et al.*, 2020).

3.2.2.12 Manganese-based fungicides

Manganese-based fungicides in nanoforms have shown significant potential. For example, MnO-NPs effectively reduced *F. wilt* in tomatoes and *V. wilt* in eggplants without affecting Mn level in plant roots or inducing phytotoxicity (Elmer and White, 2016). Similarly, manganese nanoparticles inhibited spore germination and mycelial growth rate of *C. coffeicola* in coffee seedlings (Carvalho *et al.*, 2022). Another study demonstrated that Mn₂O₃-NPs to significantly reduce disease severity in chrysanthemum transplants compared to untreated controls, indicating the potential to provide long-lasting suppression (Elmer *et al.*, 2021). Furthermore, watermelon plants treated with biosynthesized manganese NPs exhibited significant suppression of *F. wilt* disease (Noman, Ahmed, Ijaz, *et al.*, 2023). Another reported strategy for battling a variety of harmful phytopathogens in agriculture was the use of palladium-doped MnO-NPs, which had a dose-dependent inhibitory effect on mycelium growth (Vikal *et al.*, 2023).

3.2.2.13 Nickel-based fungicides

Recent researches have demonstrated the potential of nickel-based nanoparticles as strong agricultural antifungal treatment (El-Sayed *et al.*, 2023). For example, Ni_{0.5}Al_{0.5}Fe₂O₄-NPs showed remarkable antifungal action against *F. oxysporum* at a dose of 0.5 mg/ml (Sharma *et al.*, 2022). Additionally, Nickel/chitosan nanoparticles successfully suppressed *P. oryzae*-caused disease (Parthasarathy *et al.*, 2023). Furthermore, it was shown that nickel ferrite nanoparticles efficiently suppressed the mycelial growth of *D. necatrix* and *C. gloeosporioides* and that they significantly lowered the incidence of *F. wilt* (Sharma *et al.*, 2017).

Nickel-based nanoparticles exhibited two distinct effects on plant health: beneficial but also harmful. To fully utilise their potential in agricultural disease management, strategic dosing and administration approaches are essential (Sharma *et al.*, 2022).

3.2.2.14 Saccharides-based fungicides

Organic molecule-based fungicides in nanoforms have been developed in the last years and they represent an excellent alternative for standard chemical fungicides. One of the significant findings includes the interaction between glucose oxidase (GOD) and nanoparticle systems, which maintained the catalytic activity of GOD, essential for inhibiting fungal pathogens like *C. gloeosporioides* (Niu *et al.*,

2023). Moreover, nano-formulated carbendazim displayed relevant efficacy against fungal pathogens such as *F. oxysporum* and *A. parasiticus* (Sandhya *et al.*, 2017). Further research revealed that nano-conjugates like nanocarbon and flg22 significantly reduce the colonization of *Phytophthora spp.* in plants, by inducing systemic acquired resistance without directly inhibiting hyphal growth (Zhou *et al.*, 2020). Additionally, the encapsulation of bioactive agents like chitosan and β -glucans into nanoparticles has not only provided controlled release properties, but also ensured the protection of these agents from environmental degradation, enhancing their bioavailability and fungicidal activity against pathogens like *M. grisea* (Kaziem *et al.*, 2021). Furthermore, the integration of pyraclostrobin and boscalid into polymeric nanoparticles facilitated synergistic antifungal activities and reduced the toxicity in plants (Yuyang Tian *et al.*, 2022).

Research indicated that, as compared to their traditional counterparts, nano-formulations such as chlorothalonil@mesoporous silica nanoparticles with β -glucans (Kaziem *et al.*, 2021) and captan-mesoporous silica with β -glucan (Kaziem *et al.*, 2022) dramatically lowered toxicity to aquatic species, including zebrafish. Furthermore, the controlled release mechanisms of the fungicides encapsulated in nanoparticles were responsible for the decrease in toxicity by limiting the exposure to the environment and potential harm (Tang *et al.*, 2019; Yuyang Tian *et al.*, 2022)

3.2.2.15 Polymer-based fungicides

Polymer-based nano-formulations fungicides exhibit enhanced properties, including increased surface area for interaction, targeted delivery, and controlled release, thereby improving their antifungal activity against a variety of pathogens. Zineb nanoparticles encased in polymers, for example, showed better growth restriction on *A. alternata*, which was explained by an expanded contact area (Sarлак, Taherifar and Salehi, 2014). Nearly total protection was offered by nano-delivered azoxystrobin via lignin nanoparticles in seed treatments, demonstrating the efficacy and safety of these delivery methods (Kacsó *et al.*, 2022). Moreover, difenoconazole@benzoylated lignin sulfonates nanoparticles demonstrated the benefit of slow-release carriers by retaining fungicidal action on strawberry leaves for prolonged periods of time (W. Liang *et al.*, 2022). Poly(lactic-co-glycolic acid) nanoparticles, loaded with natural and synthetic antifungals, quickly penetrated *B. cinerea* conidia and hyphae, indicating a more efficient delivery and activity (De Angelis *et al.*, 2022). Extended disease control was also provided by customised polymer matrices for thiram applications (Kaushik *et al.*, 2013). A study performed by Su *et al.* demonstrated that the growth and spore generation of fungal strains were dramatically inhibited by star polycation complex (SPc)-loaded therapies, demonstrating the biocompatibility and effectiveness of SPc as a nanocarrier (Su *et al.*, 2023). Additionally, the potential for organic agriculture was demonstrated by the complexation of SPc with osthole, which increased its cytotoxicity towards pests while being safe for crops (Yan *et al.*, 2021).

The toxicity of some organic polymers-based nanomaterials was studied and reported in the literature. Animal models of lignin-derived carbon nanoparticles demonstrated little to no toxicity, suggesting their safety for wider use (El-Ganainy *et al.*, 2023). The low toxicity profile of pterostilbenes provided additional support to their beneficial usage against plant diseases (De Angelis *et al.*, 2022). Furthermore, SPc and related nanoparticles minimal toxicity emphasised their biocompatibility and showed how nanotechnology might lessen the ecological impact of pesticides while preserving agricultural productivity (Xiaodan Wang *et al.*, 2021).

3.2.2.16 Selenium-based fungicides

New developments in agricultural nanotechnology have shown how selenium nanoparticles (Se-NPs) could improve plant resistance to fungus-caused diseases. Green synthesised Se-NPs demonstrated encouraging antifungal efficacy against *Rhizoctonia solani*. A significant increase in total chlorophyll and carotenoids was seen after treatment with Se-NPs, which strengthened the plants general health and resistance to fungal infections (Hashem *et al.*, 2021). Furthermore, Se-NPs treatment increased proline content, membrane stability index, superoxide dismutase and peroxidase levels. Increased levels of

flavonoids and phenolic compounds were reported, which further supported Se-NPs protective function against illnesses and stress (Shahbaz *et al.*, 2023). Se-NPs demonstrated their effectiveness at low concentrations by efficiently suppressing the growth of *P. grisea* and fungal infection spread on tomato and chilli leaves (Joshi *et al.*, 2019). When compared to chemically synthesised Se-NPs, the biosynthesised Se-NPs from *Lactobacillus acidophilus* ML14 demonstrated greater antifungal and antiradical properties (El-Saadony *et al.*, 2021). Research on selenium nanocomposites highlighted their fungicidal activity to be comparable to commercial fungicides. Se/Ag nanocomposites demonstrated bacteriostatic and antibiofilm effects, underscoring the versatility of Se-NPs in addressing both fungal and bacterial plant diseases (Perfileva *et al.*, 2021).

3.2.2.17 Silica-based fungicides

Recent advancements in silica-based fungicides underscore how nanotechnology might improve crop protection effectiveness. SiO₂-NPs have shown promising antifungal activities against *A. solani* in eggplants (Albalawi *et al.*, 2022) and against *M. oryzae* by foliar treatment of rice plants (J. Du *et al.*, 2022), significantly altering the plants antioxidant defence mechanisms and decreasing diseases severity. Increased hydrophobicity and silica content in plant leaves, which were correlated with a lower frequency of fungal infections, demonstrated that nano-silica in maize was more effective than bulk silica (Rangaraj Suriyaprabha *et al.*, 2014). Furthermore, without appreciable changes in silver concentration, Ag/SiO₂ nanocomposite treated plants demonstrated strong resistance against *B. cinerea*, as evidenced by increased enzymatic activities and phenolic content, guaranteeing plant health with minimal risk (Baka and El-Zahed, 2022). According to research findings (Buchman *et al.*, 2019), the application of chitosan-modified silica nanoparticles (CTS-MSNs) demonstrated to inhibit *F. wilt* in watermelons, exhibiting a significant improvement in plant defences without direct nanoparticle-pathogen interaction. In parallel to this, the creation of mesoporous silica nanoparticles with N-(2-Hydroxyl) propyl-3-tri-methyl-ammonium chitosan chloride loaded with fludioxonil fungicide showed a significant decrease in the severity of disease in tomato plants, providing a sustainable substitute for conventional fungicides by permitting prolonged release and preserving efficacy without phytotoxicity (Mosa *et al.*, 2022). Furthermore, in maize tissues, captan-mesoporous silica with β-glucan demonstrated better loading efficiency, release protection under different circumstances, improved distribution, and increased inhibitory efficacy against *F. graminearum*, suggesting the possibility of targeted delivery and increased crop resilience (Kaziem *et al.*, 2022). Moreover, SiO₂@Cu nanoparticles coated with carboxymethyl cellulose exhibited remarkable antifungal efficacy against *P. capsici*, achieving complete inhibition at concentrations as low as 75 ppm after 72 hours of application (Hai *et al.*, 2021).

The toxicity of silica-based fungicides in nanoforms has been studied in recent years, and the results showed a promising biosafety profile. Using zebrafish for in vivo toxicity testing, PRO-MON-CaC was substantially less harmful than its technical equivalent, and at the dosages given, it had no discernible negative effects on rapeseed plants (Gao *et al.*, 2020). After 96 hours of therapy, CAP-MSNs-β-glucan showed 1.88 times less toxicity than captan commercial formulation, suggesting an enhanced safety margin (Kaziem *et al.*, 2022). Furthermore, disulfide-bond-bridged mesoporous organosilica-gallic acid-Fe(III) nanoparticles did not show any apparent toxicity to rice seedlings, indicating that they could be used for protecting crops without compromising the health of the plants (Y. Liang *et al.*, 2022). The toxicity of nano-pesticides, like captan@SiO₂, against bacterial strains was significantly lower than that of traditional captan pesticides. Moreover, the toxicity of these fungicides was not observed towards Gram-negative bacteria, indicating their reduced environmental impact and specificity (Sułowicz *et al.*, 2023).

3.2.2.18 Silver-based fungicides

In recent evaluations of nanotechnology potential in plant fungal disease control, silver-based nanomaterials emerged as one of the key players. In comparison to other tested nanoparticles, silver nanoparticles (Ag-NPs) exhibit dose-dependent growth inhibition, which has been demonstrated in

recent studies highlighting their strong fungicidal effects (Ibrahim *et al.*, 2020), particularly against *P. protegens* and *C. albicans* (Khan *et al.*, 2018). Ag-NPs represented a promising versatile agent against agricultural diseases such as fungal infection caused by *M. incognita*, *R. solanacearum*, and *F. oxysporum*, due to their ability to disrupt the integrity of pathogen membranes (M. Khan *et al.*, 2021). Similar outcomes were seen when testing at concentrations as high as 200 ppm for *Fusarium spp.*, where fungal growth suppression was clearly visible (Dawoud *et al.*, 2021), or even at higher concentration to obtain the total suppression of *B. cinerea* symptoms on plum fruit (Malandrakis, Kavroulakis and Chrysikopoulos, 2019). Additionally, it has been demonstrated that even lower Ag-NPs concentrations (5–20 ppm) dramatically suppressed the growth of *A. solani* (Ansari *et al.*, 2023). Even in long-term trials, Ag-NPs exhibited strong antifungal effectiveness against *B. cinerea* and *C. theae* isolates (Mythili Gnanamangai *et al.*, 2017). Significant antifungal properties have been found also for biosynthesized Ag-NPs. Ag-NPs prepared from pomegranate extract significantly limited the mycelial growth of *A. solani* and *F. graminearum* (Ibrahim *et al.*, 2020). Similarly, a green nano-formulation of Tricho-Fu21 had the capability to significantly reduce *S. rolfsii* infection (Hirpara *et al.*, 2021). Furthermore, studies also revealed that Ag₂O-NPs effectively inhibited *M. phaseolina* and *Fusarium spp.* from growing with a concentration-dependent profile (Derbalah *et al.*, 2022). Additionally, graphene oxide-silver nanocomposites were investigated for their antifungal effects. The synergy between graphene oxide sheets and Ag-NPs in the nanocomposite led to a better dispersion and consequent improved antifungal activity, with lower effective concentrations if compared to Ag-NPs alone (Chen *et al.*, 2016). Finally, novel nano-formulations such as chitosan-loaded Ag-NPs (Hoang *et al.*, 2022) and gum kondagogu-loaded Ag-NPs (Malkapur *et al.*, 2017) proved to be highly effective in controlling the severity of fungal plant diseases.

Concerns about Ag-NPs toxicity to non-target organisms, such as plants, aquatic life, and human cell lines, are raised due to their wide use into agricultural methods. In the literature, numerous researched demonstrated that Ag-NPs do not have any phyto-toxicological effects (Jo, Kim and Jung, 2009; Ahmad *et al.*, 2022; Hoang *et al.*, 2022). A plethora of published studies investigated the cytotoxicity of biosynthesized Ag-NPs and found that they could induce significant cytotoxic and genotoxic effects in various cell lines, altering mitotic indexes and cell viability in a concentration-dependent manner (Guilger *et al.*, 2017) Human umbilical vein endothelial cells were among the cell lines used to illustrate the cytotoxicity of prolonged exposure to Ag nanocomposites, such as luteolin tetraphosphate Ag-NPs, with a notable concentration-dependent susceptibility noted (Osonga *et al.*, 2020). It was also discovered that the exposure to Ag-NPs in aquatic environments caused silver to accumulate in the gills, haemolymph, and foot of mussels, along with a reduction in the amounts of Na⁺ and Cl⁻ in the haemolymph. In general, the possible ecological concerns linked to the usage of Ag-NPs regarded its bioaccumulation and physiological disruption (Tesser *et al.*, 2022). On the other hand, Ag-NPs exhibited a dose-dependent suppression of cancer cell growth against HeLa cells, with notable effects as little as 25 µg/mL (Rizwana *et al.*, 2021).

3.2.2.19 Sulphur-based fungicides

According to current state of art, the size and concentration-dependent antifungal activity of sulphur-based nanoparticles (S-NPs) make them a promising tool for the application of nanotechnology in plant pathogen control. S-NPs at 100 µg/mL demonstrated a 94.12% inhibition of fungal pathogens, which was significantly higher than that of their larger-sized equivalents (Sadek *et al.*, 2022). Similarly, tomatoes exhibit a clear nano-specific effect using S-NPs, which efficiently suppressed *F. wilt*, with an optimal dose of 100 mg/L (Cao *et al.*, 2021). It was also demonstrated that nanoparticles with a size smaller than 35 nm can inhibit the growth of whole fungi, suggesting the disruption of the fungal cell wall structure (Rao and Paria, 2013).

A published toxicology study demonstrated that S-NPs have negligible cytotoxicity against lung and kidney cells (Sadek *et al.*, 2022).

3.2.2.20 Titanium-based fungicides

Recent studies have elucidated the role of titanium-based nanomaterials in pest control for agricultural applications. The fungicide capabilities of TiO₂ nanoparticles were explored, concluding that nanometric titanium oxide is useful for crop pest protection (Pang *et al.*, 2021; Sharma *et al.*, 2023). The antifungal effectiveness of TiO₂-NPs was confirmed by showing a strong inhibition against *F. solani* (Monclou-Salcedo *et al.*, 2020). Furthermore, it was demonstrated that high concentrations (200 mg/L) of TiO₂-NPs considerably decreased the colony area of the fungal pathogen *P. pannosa* on rose leaves, exhibiting a dose-dependent antifungal action (Hao *et al.*, 2019). A similar effect of TiO₂-NPs has been reported for the inhibition of *P. protegens* (Khan *et al.*, 2018). Interestingly, green synthesis methods allowed to produce the strongest antifungal efficacy (Alabdallah *et al.*, 2023). A remarkable study performed by Nederstigt *et al.*, described a nano-formulation of carbendazim coated with TiO₂-NPS, which, when compared to conventional carbendazim treatments, not only delayed the fungicide release into aquatic systems, but also showed similar effects on macroinvertebrate communities (Nederstigt *et al.*, 2022).

Regarding the toxicity profile, despite evidences of accumulation, TiO₂-NPs did not show any additional negative consequences. However, it is recognised that elevated levels of titanium are hazardous (Pérez-Zavala *et al.*, 2022).

3.2.2.21 Zinc-based fungicides

Investigations into zinc-based nano-fungicides elucidated their significant antifungal activity. It was shown that, Zn-NPs caused hyphal rupture and the release of cellular components by efficiently inhibiting the growth of pathogenic fungi such *A. mali* and *D. seriata*, with concentration-dependent efficacy (Hilal Ahmad *et al.*, 2020). With the same dose-response profile, ZnO-NPs caused mortality in *T. castaneum* (Jahan *et al.*, 2023). At the same time, photoactivated ZnO-NPs were especially efficient against *B. cinerea*, outperforming traditional fungicides (Luksiene *et al.*, 2020). Even at concentrations as low as 0.50 µg/mL, ZnO-NPs outperformed conventional fungicides in their ability to prevent *F. oxysporum* growth (Farhana *et al.*, 2022). ZnO-NPs broad-spectrum and adaptability were shown in a real-world in-vivo application, where fruit rot disease was effectively controlled at a dose of 1.0 mg/mL (Ahmed *et al.*, 2022), or by demonstrating the wider-ranging effectiveness against a range of pathogens, such as *R. nigricans* and *P. multocida*, when compared to plant extracts (A. Farooq *et al.*, 2022). Furthermore, ZnO-NPs-lignin conjugates antifungal efficacy against *F. oxysporum* and *F. proliferatum* has been proven (Sharma and Sharma, 2023), while just a slight suppression of *S. rolfisii* growth was seen (Panichikkal, Prathap, *et al.*, 2021), indicating that the antibacterial activity of ZnO-NPs could differ depending on the pathogen strain. Moreover, it has been demonstrated that the combination of zinc and copper had synergistic effects: CuZn bimetallic nanoparticles were found to be effective against *S. cerevisiae* (Antonoglou *et al.*, 2018), while bimetallic CuZn@diethylene glycol and ZnO@polyethylene glycol nano-fibres strongly suppressed the growth of *B. cinerea* and *S. sclerotiorum* (Tryfon *et al.*, 2021). The inclusion of zinc was discovered to increase the antifungal effectiveness of the nanoparticles. Lastly, another remarkable study showed that *Terminalia bellerica* synthesised ZnO nanoparticles completely disintegrated fungal cellular structures, even at 200 ppm (Dhiman *et al.*, 2022).

Toxicity investigations demonstrated that acceptable amounts of ZnO-NPs had no negative effects on seed germination for a variety of plant species (Panichikkal, Prathap, *et al.*, 2021). Neither ZnO-geraniol nanocomposites demonstrated phytotoxicity towards host plants (Tryfon *et al.*, 2023). Furthermore, functionalised ZnO-NPs such as captan@ZnO-NPs were far less toxic than the corresponding traditional pesticide (Sułowicz *et al.*, 2023). Ongoing investigation efforts to determine non-toxic dosages for ideal plant well-being and efficient disease control, ensured that the fungicidal advantages of nanomaterials did not compromise ecological or organismal security (Dhiman *et al.*, 2022).

3.2.2.22 Zirconium-based fungicides

Zirconium oxide nanoparticles (ZrO-NPs) emerged in the last years as effective fungicides, exhibiting pronounced growth inhibition of *R. solani* and *P. versicolor*, and rivalling traditional fungicides at

substantially lower concentrations (Derbalah *et al.*, 2019; Ahmed, Ren, *et al.*, 2021). These nano-formulations improved growth, stimulated the expression of defence genes in plants, and lessen the severity of disease. Furthermore, fungicide agents release from NPs and microcapsules was faster and more controlled, which could reduce the dosage needed and mitigate its impact on the environment (Tang *et al.*, 2019).

The use of ZrO-NPs did not affect plant growth, chlorophyll content and the seedling vigour index of seedlings, unlike the conventional fungicides such as difenoconazole, exhibited toxicity at a comparable concentration (Tang *et al.*, 2019; Ahmed, Ren, *et al.*, 2021).

3.2.3 Insecticides

Insecticides are substances that have been designed to eliminate or control the number of insects that are deemed harmful, thereby safeguarding crops, livestock, and human well-being from the adverse effects of insect-transmitted diseases and destruction. They work through various mechanisms, including disrupting the nervous system of insects, inhibiting their growth, or acting as repellents to protect against insect infestation.

3.2.3.1 Terpenoid-based insecticides

Research has shown that nano-formulations have the ability to greatly enhance the bioactivity, stability, and controlled release of active components. 1,8-cineole nano-emulsion exhibited significantly enhanced bioactivity against pests, approximately doubling its effectiveness compared to the free form. This suggests that using lower doses of the nano-emulsion can be a more efficient technique for pest management (Ayllón-Gutiérrez *et al.*, 2023). Similarly, the use of nano-encapsulated *M. azedarach* extract resulted in a sustained and constant release of active components, leading to a mortality rate of up to 100% in pests such as *T. vaporariorum* and *M. persicae* (Khoshraftar *et al.*, 2020). Additionally, a study conducted on nano-emamectin benzoate-solid powder demonstrated its exceptional capacity to hydrate and penetrate difficult leaf surfaces, such as those found on cabbage. This significantly improved the pesticide's resistance to degradation and its overall efficacy when exposed to ultraviolet (UV) radiation. This phenomenon can be ascribed to the decrease in surface tension, which enables improved adherence to foliage and enhanced retention of pesticides (Gao *et al.*, 2022). Moreover, avermectin nano-delivery system exhibited exceptional storage stability and a pH-sensitive release mechanism, surpassing commercial formulations by delivering a regulated and prolonged release of avermectin (Mo *et al.*, 2021). Nanotechnology has demonstrated the ability to mitigate environmental and non-target organism hazards. spinosad sulfamic acid nanoparticles (Tian *et al.*, 2021) exhibited reduced genotoxicity towards plant cells and enhanced pest mortality rates in comparison to conventional formulations, suggesting an improvement in both safety and efficacy. The controlled release formulations provide a strategic benefit in delivering pesticides, guaranteeing a reliable and focused method for pest control (Qiu *et al.*, 2009).

The toxicity profiles of various nanomaterial-containing insecticide formulations, exhibit complex consequences depending on the specific applications and targets. Mo *et al.* reveals that avermectin nano-delivery system, when present in lower quantities, provide much greater insecticidal effectiveness compared to avermectin suspension concentrate against *Mythimna separata*. This highlights the better stability and potency of the active component when given by nanotechnology (Mo *et al.*, 2021). In contrast, Germano-Costa *et al.* suggests that nano-repellents preserve cell viability at a level exceeding 70% without significant genotoxic effects. However, they do induce an increase in cytokine expression under specific cell culture conditions, suggesting the possibility of immunological reactions (Germano-Costa *et al.*, 2022). Additional examination reveals the significant toxicity of neem urea nano-emulsion (NUNE) formulations on *A. aegypti* and *C. tritaeniorhynchus* larvae, as demonstrated by severe histopathological and biochemical changes, which are distinctly different from the effects of bulk neem oil (Mishra *et al.*, 2018). Another study demonstrates that when consumed, the double stranded RNA/branched amphiphilic peptide capsule complexes had a stronger lethal effect on pea aphids and

Tribolium larvae (Avila *et al.*, 2018). This highlights the complexes' ability to be delivered throughout the body and their effectiveness in using RNA interference to target vital proteins. These findings emphasise the delicate equilibrium between attaining precise pest control and minimising unintended consequences, which is crucial for developing safe and effective insecticidal techniques using nanomaterials.

3.2.3.2 *Polymers-based insecticides*

Nano-capsules can maintain a high mortality rate against pests like armyworm larvae for a period of 7 days, surpassing the performance of conventional methomyl solutions (Sun *et al.*, 2014). Moreover, the osthole/star polycations at the nano scale has demonstrated heightened cytotoxicity and improved pesticidal efficacy against aphids and mites. This is achieved by aiding efficient distribution into the cytoplasm through endocytosis and enhancing plant uptake (Yan *et al.*, 2021). Nano-pesticides, such as avermectin-propylene glycol alginate formulations, exhibit superior insecticidal efficacy compared to traditional solutions when targeting pests like *Plutella xylostella*. This is due to their reduced particle size and enhanced surface area. The utilisation of nanocarriers not only enhances the effectiveness of the active components, but also facilitates their widespread dispersion within plant systems, hence boosting growth even in unfavourable conditions (Wang *et al.*, 2018). The utilisation of cellulose nanocrystals in combination with thiamethoxam demonstrates a method of regulated and prolonged release, which improves the toxicity of the insecticide and reduces the necessity for repeated treatments (Elabasy *et al.*, 2020). Moreover, it has been discovered that waterborne polyurethane formulations that contain actinomycin can enhance the preservation and durability of the active ingredient on rice surfaces. These formulations exhibit resistance to environmental factors like sunlight and rainfall, indicating their potential for effective pesticide delivery (Y. Zhang *et al.*, 2020). The incorporation of UV stabilisers and transporters, such as sucrose, into abamectin nanosuspensions demonstrates a strategic method to strengthen the resistance to sunlight, decrease degradation, and boost the eco-friendliness of the formulations (Cui *et al.*, 2019). The study on imidacloprid enclosed in polymers demonstrates that the release rates can be efficiently regulated by manipulating environmental factors such as pH and temperature, providing a customised method for controlling pests (Qian, Guo and He, 2012). Furthermore, the efficacy of nano-imidacloprid against *G. pyloalis* provides additional proof of nanotechnology's ability to decrease pesticide usage and prolong control effectiveness (Memarizadeh *et al.*, 2014). The feasibility of improving the stability and penetration of genetic material for targeted pest control is highlighted by advancements in RNA-based nano-formulations, specifically dsRNA/rosin-modified polyethylene glycol and chitosan/alkyl polyglycoside. These innovations offer new opportunities for the development of environmentally friendly and effective strategies for managing pests (X. Wang *et al.*, 2023).

The osthole/star polycation combination has demonstrated enhanced absorption by strawberry plants without concurrent elevation in residue levels, suggesting its prompt biodegradation within plant cells. This compound decreases the amount of osthole residues by more than 80% compared to traditional methods, exhibiting an impressive breakdown rate of 94.05% within a five-day period after application. This guarantees minimum threats to the environment and human health (Yan *et al.*, 2021). Moreover, the dinotefuran/star polycations combination exhibited no detrimental impacts on the agronomic attributes of canola, successfully diminishing pesticide residues and emphasising its appropriateness for sustainable farming methods (Jiang *et al.*, 2022). In addition, the use of poly-succinimide nanoparticles to encapsulate avermectin preserves its toxicity even after exposure to UV irradiation. This technique effectively safeguards the active components, ensuring their effectiveness in pest management without any compromise (Su *et al.*, 2021). Nevertheless, it is important to exercise caution due to the potential toxicity hazards to aquatic species and plants that might arise from excessive concentrations of active substances. This highlights the necessity for careful handling and management (Qian, Guo and He, 2012).

3.2.3.3 Silica-based insecticides

Silica nanoparticles (SiO₂-NPs) have the potential to enhance the development and productivity of maize by interacting with NPK fertilisers, resulting in elevated levels of chlorophyll, plant height, grain yield, and protein content (El-Naggar *et al.*, 2020). SiO₂ engineered nanoparticles have been discovered to enhance planthopper resistance in rice by improving photosynthesis and water absorption (Cheng *et al.*, 2021). In addition, the larvae are more adversely affected by amorphous SiO₂-NPS obtained from olive stones and corncobs, as opposed to their crystalline counterparts. This leads to increased larval mortality and longer development durations (Idris *et al.*, 2023). The germination and growth of tomato seedlings were not affected by SiO₂-NPs nanoparticles. However, it was observed that (3-aminopropyl) triethoxysilane functionalized nanoparticles inhibited the larval growth of *Helicoverpa armigera*, indicating their potential for pest control without causing harm to the plant (Bapat, Zinjarde and Tamhane, 2020). The exposure to SiO₂-NPs had a detrimental effect on the growth of silkworm larvae, their ability to produce cocoons, and caused damage to the midgut tissue. This emphasises the importance of using caution when using SiO₂-NPs in sericulture (X. Zhang *et al.*, 2023). The field tests conducted on faba bean and soybean demonstrated that SiO₂-NPs had significant efficacy in reducing populations of diverse insect species, thereby showing their usefulness across different plant species (Thabet *et al.*, 2021). Silica nanoparticles combined with chitinase has demonstrated improved pesticidal efficacy against *Spodoptera litura*, presenting a viable and environmentally friendly substitute for conventional pesticides (Narendrakumar and Karthick Raja Namasivayam, 2021). Furthermore, the utilisation of methoxyfenozide enclosed within SiO₂-NPs has exhibited enhanced rates of release and death in pests, indicating benefits over conventional formulations (Bilal *et al.*, 2022). Fipronil-encapsulated silica nano-capsules have demonstrated potential in the control of termites, as they possess sustained release qualities that result in efficient mortality (Wibowo *et al.*, 2014).

Functionalized silica nanoparticles demonstrated minimal impact on soil chemistry, microbial communities, plant growth metrics, and animal models, indicating low environmental and physiological consequences (Karthick Raja Namasivayam *et al.*, 2023). Furthermore, the cap-like mesoporous silica@polydopamine nanoparticles had no detrimental impact on bacterial growth or cucumber seed germination, thereby validating their excellent biocompatibility (Lv *et al.*, 2023). The brine shrimp toxicity lethality assay was conducted to assess the impact on *Artemia salina*. The results showed that there was no significant mortality at different concentrations, especially at higher dosages. However, there were detected oxidative stress response mechanisms, suggesting a potential for safe ecological uses (Narendrakumar and Karthick Raja Namasivayam, 2021).

3.2.3.4 Silver-based insecticides

Silver nanoparticles (Ag-NPs) and their combinations, such as Ag-NPs mixed with lambda-cyhalothrin (Ag-NPs@L-CYN), demonstrate exceptional larvicidal properties against different pests, including *S. littoralis*, *A. aegypti*, and *H. armigera*. Concentration-dependent tests demonstrate a significant rise in death rates, with Ag-NPs and Ag-NPs@L-CYN being more potent than their conventional equivalents at lower doses (Ahmed, 2019; Kamaraj *et al.*, 2023). Additionally, biosynthesized Ag-NPs have shown enhanced antifeedant activities and significant inhibition of larval and pupal development, leading to higher mortality rates at increased concentrations (Manimegalai *et al.*, 2020). The antimicrobial and antifungal effectiveness of spherical Ag-NPs, produced from various strains, varied in their activity against prokaryotic organisms, plant pathogenic fungi, and insects. This emphasises the significance of nanoparticle morphology and synthesis method in determining their efficacy (Fouda *et al.*, 2020). Integrating silver nanoparticles into agricultural operations further improves plant growth and output. Research indicates that the application of Ag-NPs to the leaves of plants has a considerable positive impact on their development, productivity, and the quality of their fruits. This highlights the diverse advantages of nanomaterials beyond their use in controlling pests (Mosa *et al.*, 2021).

The nanoparticles produced from stearic acid showed low levels of harm to *Cyprinus carpio*, with signs of lethargy evident at concentrations over 75 mg/L and a death rate of 30% at 100 mg/L. These results

suggest that the LC₅₀ value is higher than 100 mg/L (Pavunraj *et al.*, 2020). In contrast, when acetone leaf extracts were treated with Ag-NPs, they exhibited significant insecticidal effectiveness, especially against *Sitophilus oryzae*. The results showed that the LC₅₀ and LC₉₀ values were lower for *Sitophilus oryzae* compared to *Rhizopertha domonica*, indicating that the susceptibility to products containing nanomaterials is specific to each species (Mosa *et al.*, 2021).

3.2.3.5 Chitosan-based insecticides

The study found that both chitosan and chitosan-cadmium nanoparticles showed a dose-dependent increase in mortality and antifeedant activity against *Helicoverpa armigera* and *Spodoptera litura*. Additionally, even at concentrations as low as 10 µg/mL, these substances had significant effects on mosquito longevity and fecundity. (Gabriel Paulraj *et al.*, 2017; Suriyakala *et al.*, 2023). Moreover, the utilisation of chitosan nanoparticles (CSNPs) to encapsulate lipase-double stranded RNA and chitinase-double stranded RNA has demonstrated significant efficacy in inducing larval death, impeding appropriate pupation, and interfering with gene expression in *H. armigera*. Importantly, these effects are achieved while minimising any unintended impact on non-target organisms (Kolge, Kadam and Ghormade, 2023). CNPs demonstrated increased effectiveness in killing different larval stages of *S. litura*. The efficacy of the nanocomposites was found to be dependent on the dosage, and they caused considerable disruptions in the biochemical processes of the larvae (Karthick Raja Namasivayam, Arvind Bharani and Karunamoorthy, 2018). In addition, the use of chitosan-mesoporous silica nanoparticles (CS-MSNs) has demonstrated a significant enhancement in faba bean shoot biomass and the induction of resistance against aphids. This has resulted in improved plant development and stress response, all while avoiding any direct harm to the aphids.

The use of CNPs in CNPs-double stranded RNA nano-formulations showed no harmful effects on plants while effectively reducing populations of pod borer larvae. This shows that CNPs can be biocompatible and biodegradable and possibly represent environmentally safe carriers for bio insecticidal applications (Kolge *et al.*, 2021).

3.2.3.6 Zinc-based insecticides

Nanoscale zinc oxide (ZnO) and chitosan encapsulated formulations significantly outperformed traditional treatments, demonstrating a significant decrease in egg laying, adult emergence, and pod damage (Jenne *et al.*, 2018). The combined impacts of ZnO and thiamethoxam increased the activities of superoxide dismutase and glutathione S-transferase, but also mitigated fecundity and fertility while prolonging larval and adult longevity, highlighting an improved pesticide efficacy (Jameel *et al.*, 2020). Phyto-synthesized ZnO nanoparticles have been identified as potent larvicidal and pupicidal agents against *Ae. aegypti*, reinforcing their utility in mosquito control (Chinnathambi *et al.*, 2023). Moreover, it has been discovered that ZnO nanoparticles can discourage the oviposition and decrease the ability of *S. frugiperda* eggs to hatch. This finding suggests that ZnO nanoparticles could serve as a practical substitute for traditional insecticides, as they pose a lesser likelihood of resistance development (Pittarate *et al.*, 2021). ZnO nanoparticles have demonstrated dose-dependent insecticidal activity, significantly reducing fecundity and enhancing mortality rates in *C. maculatus* (Malaikozhundan *et al.*, 2017; Malaikozhundan and Vinodhini, 2018). Furthermore, the bimetallic nanoparticles demonstrated exceptional lethality and deterrent effects on *S. frugiperda* larvae, indicating a highly favourable method for safeguarding crops with low harm to the environment (Kumaravel *et al.*, 2021).

The addition of silver and zinc nanoparticles to acetone leaf extracts resulted in a significant increase in mortality rates among rice weevils (*Sitophilus oryzae*) and lesser grain borers (*Rhizopertha domonica*). The rice weevils were found to be more susceptible to the treatments compared to the lesser grain borers. The effectiveness of these nanomaterials against the pests was measured using LC₅₀ and LC₉₀ values, which demonstrated a significant concentration-dependent toxicity (Mosa *et al.*, 2021).

3.2.3.7 Carbon-based insecticides

Carbon Quantum Dots (CQDs) conjugation and double stranded RNase gene knockdown strategies were employed to protect double stranded RNA from degradation, showing increased RNAi efficiency and stability for cellular uptake in whiteflies. This method resulted in a substantial decrease in mRNA levels of specific genes, which was associated with an increase in virus concentration and the effectiveness of transmission to plants (Kaur *et al.*, 2020). In a similar manner, the application of chitosan and CQD conjugates in larvae of the southern sugarcane borer resulted in a significant decrease in gene expression. Notably, the usage of nanoparticle-double stranded RNA complexes led to an even higher drop in gut tissues. The results emphasise the capability of formulations including nanomaterials to improve the effectiveness of insecticidal RNAi, providing a hopeful approach for controlling pests (K. Wang *et al.*, 2020).

3.2.3.8 Copper-based insecticides

The effectiveness of copper nanoparticles in managing pests, namely *S. frugiperda* larvae, has been demonstrated through many mechanisms including larvicidal activity, antifeedant effects, reduction in hemocyte levels, and suppression of acetylcholinesterase activity (Rahman *et al.*, 2022). Moreover, copper hydroxide nano-insecticides exhibit consistent performance in soil solutions, which can be related to the presence of natural organic matter. This indicates that they can be effectively used in agricultural environments (Z. Xu *et al.*, 2022). Furthermore, the biosynthesis of copper oxide nanoparticles not only effectively fights against pests that infest stored grains, but also enhances the growth of wheat plants without any harmful consequences, thereby emphasising their significance in promoting sustainable agriculture (Badawy *et al.*, 2021). Additionally, copper nanoparticles derived from entomopathogenic fungi, which are environmentally friendly, demonstrate significant effectiveness and safety in controlling mosquitoes, highlighting their ecological advantages (Vivekanandhan *et al.*, 2021). Also, the utilisation of copper nano-capsules to combat mites highlights their efficacy in reducing pest populations, hence demonstrating their versatility in various pest control situations (Dorri *et al.*, 2018).

Through a scientific investigation analysing the effects of different levels of nanoparticles on entomopathogenic fungi and maize plants, it was revealed that lower and medium concentrations did not have any negative impact on the rate at which the fungi germinated or on the physical structure of the maize plants. Furthermore, the examination of soil after the application of nanoparticles revealed no significant alterations in pH or macro- and micronutrient levels, highlighting the non-toxic nature of these nanoparticles at certain concentrations to both fungi and plants (Pittarate *et al.*, 2023).

3.2.3.9 Iron-based insecticides

The combined use of *Trigonella foenum-graecum* iron nanoparticles (Fe-NPs) and fenvalerate significantly increases the mortality rates of pests such as *S. litura* and *H. armigera*, surpassing the effects of individual treatments (Muthusamy *et al.*, 2023). Zhang *et al.* emphasises the ability of magnetic responsive carbon inlaid with iron nanoparticle to release imidacloprid in a controlled manner. This results in a substantial enhancement in the efficiency of release under alternating current conditions, leading to a reduction in pesticide residues (L. Zhang *et al.*, 2021). The formulation imidacloprid@Fe₃O₄@polydopamine@zirconium based metal-organic framework, which was released in a sustainable manner, has been found to have improved insecticidal activity and the ability to be magnetically recovered. This highlights the promise of these nano-formulations as environmentally friendly and effective options for pest management (Meng *et al.*, 2021).

3.2.3.10 Titanium-based insecticides

Application of titanium dioxide nanoparticles (TiO₂-NPs) has a substantial impact on the composition of the microbial community in soil, particularly affecting prokaryotes. However, it does not disrupt the mutually beneficial relationship between wheat roots and arbuscular mycorrhizal fungi (Moll *et al.*,

2017). Additionally, TiO₂-NPs exhibit strong insecticidal properties. The biosynthesized titanium dioxide nanoparticles (TiO₂-NPs) derived from *D. bipinnata* result in significant mortality among *A. aegypti* and *S. litura* larvae and pupae. This is accompanied by an increase in the expression of detoxifying enzymes and noticeable histological alterations (Shyam-Sundar *et al.*, 2023). TiO₂-NPs had strong larvicidal, antifeedant, and pupicidal effects in *H. armigera*, demonstrating more effectiveness compared to other treatments and causing substantial developmental defects (Chinnaperumal *et al.*, 2018).

TiO₂-NPs shown significantly reduced toxicity towards non-target organisms *T. splendens* and *E. fetida* in comparison to traditional pesticides, providing a safer option (Shyam-Sundar *et al.*, 2023). In addition, TiO₂-NPs exhibited insignificant toxicity towards earthworms, leading to an increase in their biomass and causing no impairment to soil health (Chinnaperumal *et al.*, 2018).

3.2.4 Bactericide

A bactericide is a chemical agent or substance specifically designed to kill bacteria.

3.2.4.1 Silver-based bactericides

Silver-based nanomaterials have emerged as potent bactericidal agents, showing remarkable efficacy against a wide spectrum of pathogenic bacteria. These particles, due to their ability to release silver ions, can interfere with bacterial cellular functions and enhance antibiotic sensitivity, thus offering a promising strategy to overcome drug resistance and protect agricultural crops from various diseases. The antibacterial activity of silver nanoparticles (Ag-NPs) against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* has been extensively documented (Fatima *et al.*, 2015, 2016, 2016; Osonga *et al.*, 2020; Bayat *et al.*, 2021; Mirajkar *et al.*, 2021; Ershov *et al.*, 2022; Rizwana *et al.*, 2022; Wei *et al.*, 2022). Ag-NPs have shown significant antibacterial activity, indicated by substantial inhibition zones and low minimum inhibitory concentrations, demonstrating the efficacy of Ag-NPs in suppressing bacterial growth even at low concentrations. The study by Perfilova *et al.* discusses the effect of Ag-NPs on increasing the antibiotic sensitivity of various bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*. This increased antibiotic sensitivity could be due to the action of Ag-NPs, which, through the release of silver ions, might alter bacterial membrane permeability or interfere with bacterial antibiotic resistance mechanisms (Perfilova *et al.*, 2019). Ag-NPs have also demonstrated significant efficacy against *Dickeya dadantii*, a pathogen responsible for tissue maceration in sweet potatoes. Research has shown that Ag-NPs can inhibit the growth of *D. dadantii*, as well as its swimming motility, biofilm formation, and the maceration process in sweet potato slices (Hossain *et al.*, 2019, 2023). Ag-NPs showed also significant growth inhibition of *R. solanacearum* (Haroon *et al.*, 2019; M. Khan *et al.*, 2021; Elkobrosy *et al.*, 2023), a phytopathogenic bacterium that causes brown or vascular rot disease in plants, considered one of the most destructive pathogens for agricultural crops worldwide. These antimicrobial properties, together with the root growth-promoting action, make Ag-NPs a promising treatment for plant protection. Ag-NPs represent a significant advancement in combating soil pathogens, thanks to their proven bactericidal action. According to the study in question (McGee *et al.*, 2017), the application of Ag-NPs in soil has significantly reduced the activity of essential enzymes such as dehydrogenases and ureases, which are considered fundamental indicators of microbial vitality. Moreover, Sabra *et al.* discovered that Ag-NPs, once applied to the soil, exerted a positive influence on the microbial community composition, significantly reducing the microbial biomass compared to commercial zinc and iron nanoparticles (Sabra *et al.*, 2022). Additionally, the study by Liu *et al.* examines the impact of Ag-NPs on the soil ecosystem, highlighting how they negatively influence enzymatic activity and populations of nitrifying bacteria, thus altering nitrogen transformation in the soil (X. Liu *et al.*, 2021). *Bacillus mn14*-AgNPs (Bs-Ag NPs) demonstrated potent inhibitory effects against both Gram-positive and Gram-negative bacteria, outperforming the antibiotic tetracycline in some cases (Kabeerdass *et al.*, 2021). Sotoodehnia *et al.* (Sotoodehnia *et al.*, 2019) showed that Gram-positive bacteria are more susceptible to Ag-NPs than Gram-negative bacteria. These findings underscore the potential of Ag-NPs to combat a wide range of microbial pathogens effectively.

The toxicity of silver nanoparticles as bactericides has been analysed in numerous studies. Harmful effects of Ag-NPs include potential risks of cytotoxicity towards human cells, although findings suggest relatively high biocompatibility. For instance, Ag-NPs synthesized from *Carduus crispus* showed a mild reduction in the viability of human hepatic cells HepG2 after 24 hours of treatment. However, in terms of biological safety, Ag-NPs have been found to be highly biocompatible with human skin fibroblast cells, suggesting a low risk of toxicity for direct application on the skin (Wei *et al.*, 2022). Similarly, in the study by Sudarsan *et al.*, cytotoxicity assays revealed that Ag-NPs did not induce toxicity towards either platelets or erythrocytes, indicating that these biogenic nanoparticles are non-toxic (Sudarsan *et al.*, 2021). The application of silver nanoparticles at high concentrations has shown potential toxicity (Vanti *et al.*, 2020; Adak *et al.*, 2021). For example, the application of Ag-NPs at 10% (v/v) or higher exhibited negative effects on soil microbial biomass (MBC) and enzymatic activity, reducing MBC content and the activity of dehydrogenases (DHA) and fluorescein diacetate hydrolase after 7 days. Despite some soil recovery capacity, high doses of Ag-NPs (20%) continued to have a negative impact until day 30, suggesting the need for caution in their use in agriculture. Treatment with silver nanoparticles in the concentration range between 0.5 and 10 ppm suppressed *P. syringae* symptom development, whereas concentrations above 5 ppm caused necroses and chloroses in a dose-dependent manner (Paul *et al.*, 2022). The beneficial effects of Ag-NPs include a potent antibacterial action, as demonstrated by their ability to inhibit and suppress the growth of bacteria. However, the same characteristic that gives Ag-NPs their antibacterial properties can also be a source of harmful toxic actions when these nanoparticles enter the environment; indeed, the prolonged oxidation and formation of Ag⁺ ions continue over time, enhancing the bactericidal effect but also introducing risks of toxicity to the environment and non-target organisms (Ershov *et al.*, 2022). Furthermore, the increase in radical oxygen species (ROS) production induced by Ag-NPs can be a double-edged sword, being beneficial for antibacterial activity but potentially harmful to host cells if uncontrolled, as excessive reactive oxygen species (ROS) production can also damage plant cells (Vanti *et al.*, 2020). Conversely, in the study by Namasivayam *et al.*, the application of Ag-NPs showed no significant negative effects on soil microbial population or enzymatic activity in nanoparticle treatments, nor did it adversely affect seed germination or growth parameters of *Vigna mungo* (green gram) plants, suggesting good environmental compatibility and safety for use in agriculture.

3.2.4.2 Copper-based bactericides

Copper (Cu-NPs) and copper oxide nanoparticles (CuO-NPs) are effective bactericidal agents against various pathogens, outperforming commercial pesticides in their action. The study by Varympopi *et al.* analyses the efficacy of two types of copper nanoparticles, namely Cu-NP₁ and Cu-NP₂, as bactericidal agents against various bacterial pathogens (Varympopi *et al.*, 2020). Cu-NP₂ was significantly more effective compared to reference treatments (Nordox and Kocide) and water, both at 24 and 48 hours post-inoculation. Similarly, the study by Gkanatsiou *et al.* demonstrates that Cu-NPs are effective bactericidal agents against several bacterial pathogens affecting plants, including *E. amylovora*, *X. campestris*, and *P. syringae*, with the added advantage of being applicable at lower doses than commercial pesticides (Gkanatsiou *et al.*, 2019). Indeed, the antibacterial activity of copper nanoparticles was similar to that of the commercial pesticide Kocide 2000 35 WG when applied at the registered dose of 1000 µg/mL, even though the nanoparticles were applied at significantly lower doses (10 µg/mL and 100 µg/mL). In the same vein, the study by Ma *et al.* examines the efficacy of Cu-NP incorporated into an alginate nanogel and coated with cetyltrimethylammonium chloride (CTAC) as a bactericidal agent against the pathogen *P. syringae pv. tabaci*, responsible for wild fire disease (Ma *et al.*, 2022). The Cu-NP@alginate nanogel@CTAC was more effective in controlling bacterial growth compared to the commercial bactericide thiodiazole copper, even though the total copper content in the nanogel was lower. This confirms that the nanogel offers a more efficient approach to bacterial control compared to traditional treatments. The study by Ren *et al.* confirmed that copper nanoparticles have a greater bactericidal effect compared to thiodiazole copper at significantly lower concentrations (G. Ren *et al.*, 2022). The study by Yan *et al.* explores the efficacy of copper sulphide nanoparticles (CuS-NPs) as

a bactericidal agent against the pathogen *Pseudomonas cichorii*, comparing them with the commercial copper-based bactericide known as tribasic copper chloride (TBCC). CuS-NPs showed a significant ability to reduce the number of *P. cichorii* colonies, with an inhibitory effect increasing with the concentration of CuS-NPs (W. Yan *et al.*, 2022). Compared to TBCC, CuS-NPs exhibited greater inhibitory activity, especially at low concentrations. In the same direction, the study by Ozcan *et al.* delves into the efficacy of CuS-NPs incorporated into silica particles of various sizes as bactericides, comparing them with commercial forms (Ozcan *et al.*, 2021). Those particles demonstrated high antibacterial efficacy against copper-tolerant bacterial strains, with minimum inhibitory concentrations significantly better than those observed for Kocide 3000. This suggests that incorporating Cu-NPs into silica particles enhances antibacterial efficacy compared to traditional copper formulations. The study by El-Batal *et al.* highlights the high antibacterial efficacy of CuO-NPs conjugated with streptomycin against bacterial pathogens affecting potato plants, such as *Clavibacter michiganensis*, *Dickeya solani*, and *Ralstonia solanacearum* (El-Batal *et al.*, 2020). The antimicrobial potency of the CuO-NPs-streptomycin conjugates was significantly higher compared to copper sulphate, streptomycin sulphate alone, and standard antimicrobial agents. This suggests that combining CuO-NPs with streptomycin offers synergistic advantages in eliminating bacteria. Similarly, in the study by Noman *et al.*, biogenic Cu-NPs as a bactericidal agent against bacterial fruit blotch, caused by *Acidovorax citrulli*, demonstrated significantly superior antibacterial activity compared to streptomycin (Noman, Ahmed, White, *et al.*, 2023). Two copper nanoparticles (Cu-NPs-S₁ composed 100% of copper oxide, Cu-NPs-S₂ composed of a mixture of copper oxide and hydroxide) as bactericides against *Xanthomonas campestris* pv. *vesicatoria* (Xcv), showed superior bactericidal activity compared to their respective commercial copper-based products (Varympopi *et al.*, 2022). Conversely, the study by Khan *et al.* highlights how CuO-NPs exhibited inhibitory activity against bacteria not as effective as the reference drug (Khan *et al.*, 2023). CuO-NPs showed significant dose-dependent antibacterial activity against *Escherichia coli* (I. H. Shah *et al.*, 2022; Manzoor *et al.*, 2023). Cu(OH)₂ nanorods were effective bactericidal agents against *Escherichia coli* and *Staphylococcus aureus*, with an action mechanism involving free radical production in the presence of hydrogen peroxide (Clavier *et al.*, 2022). The TiO₂/Cu₂(OH)₂CO₃ nanocomposite demonstrated high antibacterial efficiency, completely disinfecting *E. coli* cells within 80 minutes under simulated illumination (Liu *et al.*, 2020).

Several studies have reported on the toxicity of copper nanoparticles. CuS-NPs showed minimal cytotoxicity and did not induce a significant increase in apoptosis levels on human L02 hepatic cells (W. Yan *et al.*, 2022). Similarly, toxicity studies on local systemic pesticide using copper as one of the main antimicrobial agents showed relatively low toxicity towards human L02 hepatic cells. Furthermore, no phytotoxicity was observed on tomato plants treated with locally systemic pesticide particles, indicating that the treatment is safe for plants (Ozcan *et al.*, 2021). The phytotoxicity of nanoparticles was also investigated for the mixed valence copper loaded silica nanogel (MV-CuSiNG) on *Vinca sp.* and *Hamlin orange*. *Vinca sp.* showed moderate-high levels of tissue damage when exposed to MV-CuSiNG, while *Hamlin orange* exhibited strong tolerance to copper-induced phytotoxicity, even at the highest concentrations tested (Young and Santra, 2014). Sharma *et al.* investigated the ecotoxicity of Cu-NPs on two bacterial strains, the Gram-positive *Bacillus subtilis* and the Gram-negative *Pseudomonas fluorescens* (Sharma, Goyal and Chudasama, 2021). The results showed greater ecotoxicity on the Gram-positive bacteria *B. subtilis* compared to the Gram-negative *P. fluorescens*. Moreover, with an increase in the hydrodynamic sizes of the Cu-NPs, ecotoxicity decreased in the case of the Gram-negative strain *P. fluorescens*, while a systematic size dependency was not observed for the Gram-positive strain *B. subtilis*.

3.2.4.3 Zinc-based bactericides

Zinc oxide nanoparticles (ZnO-NPs) as bactericidal agents have shown significant efficacy in reducing biofilm formation. Studies by Ishwarya *et al.* (Ishwarya *et al.*, 2018) and Saqib *et al.* (Saqib *et al.*, 2022) highlight how ZnO-NPs can inhibit biofilm formation by both Gram-positive and Gram-negative bacteria. The study by Jahan *et al.* (Jahan *et al.*, 2023) confirms the bactericidal action of ZnO-NPs against Gram-positive and Gram-negative bacteria, particularly *Clavibacter michiganensis* and *Pseudomonas syringae*,

with the inhibitory effect of ZnO-NPs being comparable to that of ciprofloxacin, a reference antibiotic. Similarly, Fan *et al.* (Fan *et al.*, 2023) emphasizes the ability of ZnO-NPs to disrupt the balance of various chemical, physical, and biological processes around bacteria, thereby destroying their ability to form biofilms, especially against *Pseudomonas syringae pv. tabaci*, a pathogen causing "wildfire" disease in tobacco. It is noted that ZnO-NPs exhibited greater toxicity towards this bacterium compared to bulk zinc oxide or deionized water. The antibacterial efficacy against *Pseudomonas syringae pv. tabaci* by the graphitic carbon nitride with zinc oxide nanoparticles composite was also demonstrated by Cai *et al.* (Cai *et al.*, 2023). In the same direction, the study by Ahmed *et al.* demonstrates the efficacy of biogenic ZnO-NPs against two types of phytopathogenic bacteria, *Burkholderia glumae* and *Burkholderia gladioli*, in reducing not only bacterial growth but also their ability to form biofilms (Ahmed, Wu, *et al.*, 2021). Hsueh *et al.* report that ZnO-NPs significantly affect the biofilm formation capability of the bacterium *Bacillus subtilis* (Hsueh *et al.*, 2015). Wild-type strains of bacteria can form thin biofilms under low concentrations of ZnO-NPs (5 and 10 ppm), but biofilm formation is inhibited at concentrations above 25 ppm. The study by Wang *et al.* describes the antibacterial activity of zinc oxide quantum dots (ZnO-QDs) against *Acidovorax citrulli*, a pathogen causing diseases in melon plants (H. Wang *et al.*, 2023). ZnO-QDs adhere and cluster around the surface of bacteria, slowly releasing zinc ions and generating significant amounts of hydroxyl radicals under light irradiation, penetrating the cell membrane and causing an increase in internal radical oxygen species levels, leading to DNA damage and destruction of the bacteria's internal defence systems.

No significant signs of toxicity were detected for zinc nanoparticles as bactericides. Thiazole-Zn nanoparticles proved to be effective bactericides against various bacterial pathogens compared to the commercial pesticide formulation. Moreover, the thiazole-Zn nano-formulation also showed lower cytotoxicity compared to the commercial pesticide formulation (Xiao *et al.*, 2019). The study by Panichikkal *et al.* demonstrated no effect for *Pseudomonas sp.* DN18 when treated with various concentrations of ZnO-NPs and salicylic acid (SA). However, a weak inhibition on the growth of *S. rolf sii* with different concentrations of ZnO-NPs and SA solutions was observed compared to the control (Panichikkal, Prathap, *et al.*, 2021). Similarly, in the study by Boddupalli *et al.*, no biocidal response was observed towards *P. striata* 27 and *A. chroococcum* W5 when exposed to ZnO-NPs concentrations of 25–75 mmol/L. With increasing doses of nanoparticles, the *P. striata* 27 strain showed a decrease in population, indicated by the difference in optical density. This suggests that ZnO-NPs have an inhibitory effect on this strain at higher concentrations. Conversely, the *A. chroococcum* W5 strain showed an increase in optical density with increasing nanoparticle concentration, indicating a possible tolerance or even a stimulating effect of ZnO-NPs on this strain (Boddupalli *et al.*, 2017).

3.2.4.4 Silver, zinc oxide and copper nanoparticles against *Xanthomonas oryzae pv. oryzae* (Xoo)

Silver, zinc oxide and copper nanoparticles have been extensively researched for their antibacterial properties against the pathogen *Xanthomonas oryzae pv. oryzae* (Xoo), which causes rice blast disease, one of the most devastating diseases for rice crops worldwide. Studies show that Ag-NPs effectively inhibit bacterial growth and biofilm formation of Xoo in a dose-dependent manner, with significant activity observed even at low concentrations. Ag-NPs at a dose of 5 µg/mL were effective, achieving maximum biofilm inhibition at 50 µg/mL due to bactericidal activity (Mishra *et al.*, 2020). Another research highlighted that Ag-NPs synthesized from *Lantana camara L.* flower exhibit high antibacterial and antibiofilm activity against *Ralstonia solanacearum* and Xoo, with 10.0 µg/mL showing the highest effectiveness (Cheng, Wang and Zhang, 2020). Botanical Ag-NPs were found to possess strong bacteriostatic activity against the Xoo strain C2 at 20 µg/mL, significantly reducing bacterial numbers in liquid broth (Ye Tian *et al.*, 2022). Biosynthesized Ag-NPs showed strong antibacterial activity against Xoo strain LND0005 and Ao strain RS-1, significantly inhibiting biofilm formation, swimming motility, damaging bacterial cell walls, and promoting rice seedling growth (Ibrahim *et al.*, 2019). Furthermore, a composite of graphene oxide and silver nanoparticles (GO-Ag) demonstrated superior antibacterial activity against Xoo strains compared to Ag-NPs alone and was significantly more effective than GO without Ag-NPs (Liang, Yang and Cui, 2017).

ZnO-NPs possess pronounced antibacterial activity against Xoo, attributed to their relatively small size and high surface area-to-volume ratio (Shobha *et al.*, 2020). Ogunyemi *et al.* highlight the efficacy of biologically synthesized ZnO-NPs as potent bactericidal agents against the Xoo strain GZ 0003, demonstrating their potential to inhibit bacterial growth, reduce biofilm formation, and compromise the viability and motility of pathogenic bacteria (Ogunyemi *et al.*, 2019). Biologically synthesized ZnO-NPs were effective in inhibiting the growth of Xoo GZ 0005, with a greater effect compared to bulk chitosan and bulk ZnO, as well as chitosan nanoparticles (Abdallah *et al.*, 2020). Similarly, thiazole-Zn nanoparticles were shown to have superior antibacterial activity against Xoo compared to the commercial pesticide formulation (Xiao *et al.*, 2019).

Cu-NPs demonstrated effective antibacterial activity against Xoo at much lower concentrations than commonly used copper formulations, such as copper oxychloride, copper hydroxide, and Bordeaux mixture (Datta Majumdar, Ghosh and Mukherjee, 2021). Molybdenum sulphide and copper nanoparticles (MoS₂-Cu-NPs) were shown to completely inhibit the growth of Xoo at significantly lower concentrations (62.5 µg/mL) compared to Cu-NPs (250 µg/mL) and Kocide 3000 (1200 µg/mL), indicating a higher antibacterial efficiency of MoS₂-Cu-NPs (Y. Li, Liu, *et al.*, 2020).

3.2.5 Abiotic or biotic stress tolerance enhancers

Abiotic or biotic stress tolerance enhancers refer to compounds or genetic alterations that help plants in enduring environmental stresses that are either non-living in nature (abiotic), such as drought, salinity, and severe temperatures, or induced by living organisms (biotic), such as bacteria, viruses, and fungus. These enhancers optimise a plant's capacity for resistance and sustain productivity in unfavourable situations by increasing its physiological and biochemical reactions.

3.2.5.1 Zinc-based abiotic or biotic stress tolerance enhancer

Zinc-based nanomaterials (Zn-NMs) play an important part in enhancing the ability of plants to withstand different environmental and biological stresses. Zinc sulphide nanoparticles (ZnS) and ZnO nanoparticles have been demonstrated to affect the physiological and biochemical traits of plants, hence aiding in stress alleviation. ZnS-NMs caused a notable decrease in carotenoids and chlorophyll b levels in cucumber plants, indicating a stress reaction. However, this did not affect the overall plant biomass or cause any obvious signs of toxicity (Song *et al.*, 2019). Moreover, ZnO nanoparticles had a crucial role in enhancing the process of seed germination and improving the growth of rice seedlings when they were exposed to arsenic stress. This emphasises the potential of ZnO nanoparticles in mitigating the harmful effects of toxic metals (Wu *et al.*, 2020). The presence of these nanoparticles resulted in an increase in chlorophyll concentration and a significant decrease in arsenic uptake, thus protecting plants against the harmful effects of heavy metal toxicity. In addition, the combination of ZnO nanoparticles with betaine or proline, as observed in coriander plants, greatly enhanced growth characteristics when exposed to high salt levels. This emphasises the cooperative impact of Zn nanoparticles with organic compounds in mitigating stress (Hanif and Zia, 2023; Hanif *et al.*, 2023). Additional evidence was provided by research conducted on wheat and rice, which demonstrated that the use of nano-materials such as SiO₂-NPs and ZnSO₄-NPs significantly boosted grain yields and improved nutrient recovery efficiency. This shows the efficacy of these nanoparticles in enhancing crop productivity regardless of varying environmental pressures (Ahmed *et al.*, 2023; Ali, Ibrahim and Omer, 2023). Utilising Zn-NPs in mulberry cuttings resulted in greater resistance to drought, as demonstrated by increased rates of survival, growth of shoots and roots, and decreased levels of oxidative stress indicators (Haydar, Kundu, *et al.*, 2023). Likewise, ZnO-NPs obtained from *U. lactuca* have remarkable photocatalytic characteristics, which aid in the breakdown of pollutants and prevention of biofilm formation. As a result, these nanoparticles have promising uses in environmental cleanup and pathogen management (Ishwarya *et al.*, 2018).

3.2.5.2 *Silica-based abiotic or biotic stress tolerance enhancer*

Silicon-based nanoparticles (Si-NPs) can effectively improve plant resilience to both abiotic and biotic stresses. Research has demonstrated that Si-NPs can enhance plant development, increase biomass accumulation, and improve chlorophyll content in the presence of heavy metal pollution and drought stress. This was observed in wheat plants treated with Si-NPs at a concentration of up to 100 mg/kg in soil contaminated with cadmium (Khan *et al.*, 2020). In addition, the utilisation of Si-NPs, along with bacterial inoculation, effectively improved the adverse effects of lead stress in coriander. This resulted in increased biomass and chlorophyll content, while simultaneously decreasing oxidative stress markers (Fatemi, Esmail Pour and Rizwan, 2020). The supplement of SiO₂-NPs in barley effectively reduced the negative effects of cadmium stress. This resulted in enhanced growth of both roots and shoots, as well as a decrease in the concentration of heavy metals. Furthermore, there were improvements in chlorophyll and soluble protein content (He *et al.*, 2023). Furthermore, the utilisation of Si-NPs resulted in enhanced growth and decreased oxidative stress in sugar beets when subjected to water scarcity situations (Namjoyan *et al.*, 2020). The application of Si in maize resulted in a significant increase in both biomass and chlorophyll content. Further, Si treatment boosted the plant's ability to resist herbivore stress, suggesting an enhanced defensive capacity achieved through the upregulation of defence-related genes and lignin production (Xiao *et al.*, 2023). Moreover, laboratory experiments have demonstrated that SiO₂-NPs can alleviate the negative impacts of water scarcity stress by increasing chlorophyll levels and decreasing oxidative damage (Mahmoud *et al.*, 2020). Also, the utilisation of nitrogen-based fertiliser and nano-materials demonstrated a substantial enhancement in wheat grain yield and nitrogen recovery efficiency under various environmental circumstances. This highlights the promising prospect of incorporating nano-materials with traditional fertilisation methods (Ali, Ibrahim and Omer, 2023).

3.2.5.3 *Silver-based abiotic or biotic stress tolerance enhancer*

The application of Ag-NPs greatly enhances the ability of rice plants to resist diseases and tolerate cold temperatures. This improvement is achieved by a technique called "stress training," which involves treating the seeds and leaves of the plants with Ag-NPs prior to their exposure to stressors. This approach not only provided higher resistance but also did not impair production, indicating a viable option for crop protection without reducing agricultural output (Chen *et al.*, 2023). Additional investigation has uncovered that citrate-capped Ag-NPs, particularly when used in conjunction with indole acetic acid, effectively mitigate the negative effects of saline stress on maize. This leads to improved growth metrics and enhances photosynthetic efficiency (Afridi *et al.*, 2023). Moreover, one other study demonstrated that biosynthesized Ag-NPs effectively bolstered plant immunity against the pathogen *A. brassicicola* (Kumari *et al.*, 2020). This was achieved by regulating early defence responses and stimulating the production of antimicrobial chemicals. Furthermore, the addition of Ag-NP has been found to reduce oxidative stress in wheat when grown in saline circumstances, leading to enhanced germination rates and increased antioxidant activity (Wahid *et al.*, 2020). Ag-NPs have also demonstrated effectiveness in mitigating the negative effects of mercury stress on fenugreek plants, enhancing stress tolerance markers such as proline and hydrogen peroxide content (Khalofah, Kilany and Migdadi, 2021).

3.2.5.4 *Selenium-based abiotic or biotic stress tolerance enhancer*

Research has been conducted on selenium-based nanoparticles (Se-NPs) to explore their ability to enhance plant tolerance to abiotic stress, providing insights into mechanisms of action and outcomes on plant health and productivity. The application of CeO₂-NPs and Se-NPs had an impact on the cellular structures of mung beans. Specifically, it altered the integrity of vacuoles and the size of starch granules. Additionally, it influenced the formation of seed pods and the yield of grains (Kamali-Andani *et al.*, 2023). Similarly, the use of Se-chitosan-NPs demonstrated potential in reducing the negative effects of salt stress in bitter melon (Sheikhalipour *et al.*, 2023). This was achieved by improving growth metrics, enhancing chlorophyll fluorescence, and regulating antioxidant enzyme activities. This was also corroborated by gene expression analysis demonstrating enhanced stress responses. Furthermore, it

was discovered that Se-NPs decreased the accumulation of cadmium in coriander plants. This led to improvements in both shoot and root growth, as well as increased chlorophyll content and antioxidant capacity, particularly when the plants were subjected to cadmium-induced stress (Babashpour-Asl, Farajzadeh-Memari-Tabrizi and Yousefpour-Dokhanieh, 2022). On top of that, Se-NPs mitigated the oxidative stress caused by arsenite in soybean roots by enhancing antioxidant defences and activating stress-responsive genes (Zeeshan *et al.*, 2023).

3.2.5.5 Iron-based abiotic or biotic stress tolerance enhancer

Iron-based nanoparticles, such as Fe₂O₃-NPs and nano-maghemite, have been demonstrated to regulate important physiological factors. These include decreasing the absorption and movement of sodium, improving the ratio of potassium to sodium, and promoting the intake of essential nutrients and antioxidants. In particular, the application of Fe₂O₃-NPs has effectively decreased the amount of salt in the roots of ajowan plants grown under saline circumstances. This treatment has also been linked to enhanced accumulation of osmolytes, increased activity of antioxidant enzymes, and higher content of essential oils in the plants (Abdoli, Ghassemi-Golezani and Alizadeh-Salteh, 2020; Ghassemi-Golezani and Abdoli, 2021). Furthermore, the application of iron nanoparticles has led to increased plant height, spike length, and biomass in wheat under normal and drought stress conditions, indicating enhanced growth and stress tolerance capabilities (Adrees *et al.*, 2020). Furthermore, the application of nano-maghemite priming has been discovered to promote the growth and productivity of stressed seedlings by improving the absorption of essential micronutrients and decreasing the accumulation of fluoride (Banerjee and Roychoudhury, 2021).

Treatments with greater concentrations of iron-based nanoparticles (4,000–8,000 mg kg⁻¹) caused genotoxicity in maize seedlings. This was observed by reduced fresh weight, disturbances in the cell cycle, and alterations in DNA banding patterns (Youssef *et al.*, 2021).

3.2.5.6 Copper-based abiotic or biotic stress tolerance enhancer

Exposure to nano copper (Cu-NPs) and copper oxide nanoparticles (CuO-NPs) resulted in elevated copper accumulation in plants, which had a negative impact on some physiological parameters. For instance, it caused a reduction in photosynthesis and biomass in cucumber (Huang *et al.*, 2019), and a decrease in shoot biomass in rice (Wang, Sun and Ma, 2019). Nevertheless, these compounds also altered the antioxidant responses, suggesting a plausible protective mechanism against stress. Moreover, it has been discovered that biogenic Cu-NPs have a substantial impact on increasing salinity tolerance in maize. This leads to improved growth and the reconfiguration of ionic homeostasis (Noman *et al.*, 2021).

3.2.5.7 Chitosan-based abiotic or biotic stress tolerance enhancer

Studies have demonstrated that chitosan-based nanoparticles (CS-NPs) and chitosan-salicylic acid nanocomposites (CS-SA-NCs) greatly improve plant tolerance and physiological performance when plants are exposed to salinity stress. CS-NPs priming in rice successfully alleviated the negative impacts of salinity. This resulted in improved germination parameters, seedling growth, chlorophyll content, and reduced oxidative stress, through the modulation of antioxidant enzyme activities and proline content (Mosavikia *et al.*, 2020). Similarly, when grapes were exposed to high salt levels, applying CS-SA-NCs directly to the leaves, it increased the amount of photosynthetic pigments, boosted the levels of soluble proteins and carbohydrates, and decreased the leakage of electrolytes, illustrating the protective role of these nanocomposites against salt-induced oxidative damage. In addition, the application of CS-SA-NCs was discovered to improve the nutrient imbalance resulting from salinity, enhancing the content of essential nutrients such as nitrogen, phosphorus, potassium, magnesium, iron, and zinc, and regulating the Na⁺/K⁺ ratio, thereby maintaining ionic homeostasis in stressed plants (Aazami *et al.*, 2023).

3.2.5.8 Carbon-based abiotic or biotic stress tolerance enhancer

Carbon dots (CDs) and carbon quantum dot nanoparticles (CQDs), including those that have been modified with proline (Pro-CQDs-NPs), were found to greatly improve both plant stress resistance and growth. CDs alleviated the adverse effects of 2,4-dichlorophenoxyacetic acid, its sodium salt, and NaCl stressors on rice by reducing oxidative stress and improving nutrient absorption. This led to an increase in biomass and chlorophyll content (Y. Li, Gao, *et al.*, 2020). Similarly, in wheat, CDs through irrigation and spraying resulted in changes in the accumulation of tellurium, specifically increasing the iron content in both the roots and shoots, while decreasing the accumulation of cadmium. This process effectively enhances the biofortification of iron in grains (Zhu *et al.*, 2023). Pro-CQDs-NPs shown significant efficacy as priming agents in grapevines, mitigating the negative effects of salinity stress, improving leaf growth, photosynthetic efficiency, and antioxidant responses, while decreasing stress indicators such as electrolyte leakage and malondialdehyde (Gohari *et al.*, 2021). For wheat, nanocomposites were shown to enhance germination parameters and seedling vigour, particularly under salinity stress, by improving root and shoot growth, and increasing germination speed and percentage, highlighting their potential in improving crop resilience and yield under abiotic stresses (Haydar, Ali, *et al.*, 2023).

High concentrations of CQDs exhibit toxicity or lack of efficiency, emphasising the significance of regulating the dosage when utilising them in nanomaterial-based products to promote stress tolerance (Gohari *et al.*, 2021).

3.2.6 Herbicides

The herbicides are chemical substances designed to control or eliminate unwanted vegetation. They play a crucial role in agricultural and landscape management by selectively targeting weeds that compete with crops or mar aesthetic landscapes.

3.2.6.1 Polymer-based herbicides

The following studies have elucidated the performance of organic polymer-based herbicides in nanoforms, highlighting the improvements of nanoparticles compared with conventional formulations. For example, nanocomposites based on essential oils with arabic gum-gelatin and apple pectin cross-linked with citric acid have demonstrated a capability to suppress the growth of unwanted plants similar to that of metribuzin, a commonly used chemical herbicide (Taban, Saharkhiz and Kavooosi, 2021). Similarly, water-dispersible powders formulated with nanotechnology containing an herbicide, in controlling the weed *Echinochloa colona* in rice crops, showed performances comparable to those of the commercial herbicide Satunil[®] (Lim *et al.*, 2022). The efficacy and safety of a new nanotechnological formulation, Metolachlor-loaded Zeolitic Imidazolate Framework-8 (METO@ZIF-8), in controlling barnyard grass, was found to be comparable to conventional METO formulations (L. Ren *et al.*, 2022). Disulfide aminophenoxazinone compound complexed with gamma-cyclodextrin (DiS-NH₂-γ-CD) and the polymeric nanoparticles DiS-NH₂ have shown an increase in herbicidal activity compared to traditional applications (Mejías *et al.*, 2023).

In terms of toxicity, metolachlor (METO), an amide herbicide encapsulated within the zeolitic imidazolate framework-8 (ZIF-8), showed high efficacy in controlling barnyard grass and lower toxicity compared to METO for both corn (a non-target species) and zebrafish (an aquatic toxicity model), suggesting a reduction in environmental harm risk and to non-target species (L. Ren *et al.*, 2022). In the study by Kumar *et al.*, herbicide-loaded pectin nano-capsules demonstrated reduced cytotoxicity compared to the original herbicide (Kumar *et al.*, 2017). In field trials, these nano-capsules effectively controlled weeds with a lower herbicide dosage and minimal toxicity, without harming wheat crops. Diyanat *et al.*'s research highlighted the negative impact of the commercial formulation of pretilachlor on the mitotic index of onion cells, with an increase in chromosomal anomalies as the concentration increased (Diyanat *et al.*, 2019). In contrast, the nano-formulation showed a reduction in chromosomal aberrations, suggesting that encapsulation can decrease DNA damage.

3.2.6.2 Silica-based herbicides

Silica-based nanoform herbicides have demonstrated their potential to enhance the delivery and efficacy of agricultural chemicals. For instance, uniconazole-loaded porous hollow silica nanoparticles (PHS-NPs) significantly improved the controlled release and bioavailability of herbicides, resulting in notable growth retardation in rice seedlings (Tan *et al.*, 2012). Similarly, it has been demonstrated that Si-NPs augment biomass, enhance lignin biosynthesis, and improve photosynthetic efficiency in tomato plants, thereby successfully reducing the adverse effects of *Orobanche ramosa* infection (Madany *et al.*, 2020). Additionally, Si-NPs facilitated a marked accumulation of silicon in pea seedlings, reducing the severity of *Phelipanche* infection and promoting lignin formation for cell wall defence, thereby supporting plant growth under biotic stress (Shabbaj *et al.*, 2021).

3.2.6.3 Atrazine-based herbicides

Nanoparticle-based formulations of atrazine, such as atrazine nano-formulations (Preisler *et al.*, 2020), poly(lactic-co-glycolic acid) nanoparticles loaded with atrazine (Schnoor *et al.*, 2018) and essential oil-based nano-enabled formulation as a nanocarrier system atrazine (Kumar, Kanwar and Mehta, 2022), have shown remarkable efficacy in inhibiting plant growth. Atrazine nano-formulations impede the growth of soybean and *Bidens Pilosa*, compared to control groups and not nano-formulated counterparts. Poly(lactic-co-glycolic acid) nanoparticles with encapsulated atrazine exhibited even stronger effects on plant roots, enhancing herbicidal action at lower doses. Atrazine essential oil-based nano-enabled formulation provides exceptional herbicidal activity even at lower concentrations than its commercial counterparts.

A comprehensive risk assessment framework for nano-atrazine in soil medium has been established, revealing minimal risk to occupational workers through oral and dermal exposure. The maximum calculated application rate of atrazine in nanocarriers without posing human health risks was determined to be 0.63 kg/ha (Shahane and Kumar, 2023).

3.2.6.4 Glyphosate-based herbicides

Advancements in nanotechnology have significantly enhanced the application and efficiency of glyphosate through innovative formulations and delivery systems. For instance, a nanocomposite named GO–GLY, comprising graphene oxide (GO) and glyphosate (GLY) (Wang *et al.*, 2022), has demonstrated superior inhibitory effects on the growth of wheat and rape seedlings compared to the use of either GO or GLY alone. In a similar vein, GLY has been incorporated into pH-responsively controlled-release pesticide system (Xiang *et al.*, 2017). This system employs a magnetic nanocarrier made from diatomite and Fe₃O₄ to deliver glyphosate directly to the target weeds. The high adhesion capability of pH-responsively controlled-release pesticide system on weeds, combined with its controlled-release performance, improves the effectiveness of herbicides, surpassing that of commercial GLY formulations.

3.2.6.5 2,4-dichlorophenoxyacetic acid-based herbicides (2,4-D)

Research in nanotechnological materials has led to significant advancements in optimizing the controlled release of herbicides, with a particular focus on 2,4-dichlorophenoxyacetic acid (2,4-D). A study examines the capability of a nanosized rice husk (n-RH) nano-sorbent to sustain the release of 2,4-D over several days without polymer coatings, underscoring its eco-friendly benefits and selective herbicidal efficacy, as evidenced by no adverse effects on non-target plants (Abigail M, Samuel S and Chidambaram, 2016). Concurrently, another study revealed that carboxymethylchitosan-(7-diethylaminocoumarin-4-yl) methyl succinate micelles efficiently release the 2,4-D herbicide under simulated sunlight, an effect enhanced by the photocleavage of coumarin groups. Additionally, the controlled release of herbicides like 2,4-D and picloram from silica-based materials has demonstrated improved delivery and efficiency (Prado, Moura and Nunes, 2011). Additionally, perylene-2,4-D nano-

herbicide facilitate the controlled in planta release of 2,4-D, resulting in significant improvements in the inhibition of root and shoot growth (Atta *et al.*, 2015).

3.2.6.6 Other herbicides

A study has shown that plants like duckweed (*landoltia punctata*) exposed to higher concentrations of Ag-NPs accumulate silver levels above the WHO drinking water safety limits, presenting a significant risk to aquatic ecosystems when released (Lalau *et al.*, 2020).

3.2.7 Nematicides

Nematicides are substances, either chemical or biological, that are employed to manage or eradicate nematodes, which are parasitic worms capable of inflicting substantial harm to plants in the fields of agriculture and horticulture.

3.2.7.1 Silver-based nematicides

Researches on silver-based nanoparticles (Ag-NPs) have revealed their effectiveness in fighting root nematodes, providing a possible substitute for traditional nematicides with improved functionality. El-Batal *et al.* have shown that using Ag-NPs and silver-boron nanoparticles (AgB-NPs) as treatments can greatly increase the death rate of *Meloidogyne incognita juveniles*. These improvements are shown over time and with different concentrations of the nanoparticles (Elkobrosy *et al.*, 2023). Significantly, treatments employing Ag-NPs obtained from *Ficus sycomorus* and nanoparticles synthesised using green methods not only decreased nematode survival but also demonstrated significant antimicrobial activity against pathogens such as *Ralstonia solanacearum*, suggesting a wide-ranging bioactivity (El-Batal *et al.*, 2019). In vitro assays highlighted that concentrations as low as 0.1 µg/ml of Ag-NPs could cause the death of all nematodes. This demonstrates the remarkable effectiveness of Ag-NPs at very low doses (Baronia *et al.*, 2020). Moreover, in live animal experiments, it was discovered that biogenic nanoparticles and extracts derived from *Cladophora glomerata* effectively impeded the ability of *Meloidogyne javanica* eggs to hatch and restricted the movement of juvenile nematodes. This resulted in improved plant growth and the activation of systemic acquired resistance by increasing the expression of defence-related genes and enzyme activities (Ghareeb *et al.*, 2020). In addition, the use of Ag-NPs on infected tomato and eggplant cultivars led to enhanced plant vigour, decreased nematode reproduction metrics, and altered genetic material in treated plants. This indicates a comprehensive approach to nematode management (Abdellatif, Abdelfattah and El-Ansary, 2016). Green synthesized Ag-NPs demonstrated no phytotoxic effects, indicating their safe application in agricultural practices (Danish *et al.*, 2022).

3.2.7.2 Zinc-based nematicides

Cu-doped ZnO nanoparticles (NPs) have a dose-dependent inhibitory effect on fungal pathogens, including *Botrytis cinerea* and *Sclerotinia sclerotiorum*, as well as nematodes, specifically *Meloidogyne javanica*. These nanoparticles are more effective than conventional Cu@poly(ethylene glycol) nanoparticles (Tryfon *et al.*, 2022). Moreover, the utilisation of ZnO-NPs as a spray on carrot crops has demonstrated a substantial improvement in plant growth characteristics, chlorophyll levels, and carotenoid content. At the same time, it effectively decreases root galling and the multiplication of nematodes (Ahamad and Siddiqui, 2021). The ZnO-NPs nanofluid exhibited significant antibacterial activity and also induced notable nematocidal effects, such as cuticle deformation and mortality in *M. incognita*, without any negative impact on plant seed germination and growth (Kalaba *et al.*, 2021). Moreover, the utilisation of ZnO-NPs in conjunction with oxamyl has been recognised as a remarkably efficient method for managing *M. incognita juveniles* and minimising root galls. This approach demonstrates superior efficacy when compared to individual treatments of bulk ZnO and oxamyl (El-Ansary, Hamouda and Elshamy, 2022).

3.2.7.3 Chitosan-based nematicides

The primary application of chitosan nanoparticles was in conjunction with avermectin to assess its nematicide impact. Studies demonstrate that nano-encapsulated nematicides give improved functionality, such as temperature-sensitive release mechanisms, enhanced photostability, and better distribution and uptake by target nematodes. Consequently, these advancements result in heightened efficacy when compared to conventional formulations. The avermectin@quaternary ammonium chitosan surfactant nano-capsules displayed enhanced adherence to crop leaves and demonstrated controlled release characteristics. This optimised the management of aphids by adapting to changes in ambient temperature (H. Chen *et al.*, 2022). In the same way, avermectin@benzil-modified chitosan oligosaccharide nano-capsules responded to reactive oxygen species, which helped in releasing the contents in a precise manner. These nano-capsules also showed better resistance to light and were distributed effectively within the bodies of nematodes. As a result, they enhanced their ability to kill nematodes while causing less harm to non-target organisms such as earthworms (L. Li *et al.*, 2023). The use of avermectin-chitosan/poly- γ -glutamic acid nanoparticles resulted in a notable rise in mortality rates among nematodes. This was achieved by improving the dispersion of the nanoparticles in water and delivering them directly to the target organisms (Liang *et al.*, 2018).

3.2.7.4 Copper-based nematicides

The introduction of copper oxide nanoparticles (CuO-NPs) was observed to have a negative impact on *Meloidogyne incognita*, as it inhibited the hatching of eggs and considerably impeded the growth of *Pythium vexans* (A. Khan *et al.*, 2022; Khan *et al.*, 2023). Moreover, these nanoparticles significantly increased the growth of chickpea plants by mitigating root infections and enhancing physiological characteristics. The growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum* was inhibited by Cu-doped nanoparticles in a dose dependent manner. Also, these nanoparticles induced paralysis in *Meloidogyne javanica*, resulting in a decrease in galling and nematode population (Tryfon *et al.*, 2022).

Copper-based nano-formulations present an eco-friendly approach, effectively controlling pathogens without phytotoxic effects on lettuce, highlighting their potential for sustainable agriculture.

3.2.7.5 Silica-based nematicides

Silica nanoparticles (Si-NPs), like CuO-NPs, exhibit strong nematocidal properties against root-knot nematodes (*Meloidogyne incognita*), leading to a considerable decrease in egg hatching and juvenile mortality on eggplant (El-Ashry *et al.*, 2022). Si-NPs, when used in conjunction with lower doses of commercial nematicides, enhance the inhibition of nematode reproduction and the gall formation. In addition, the application of SiO₂-NPS to the leaves improves the growth characteristics, chlorophyll levels, and carotenoid content in carrots. This effect is greater than that observed with ZnO-NPs and TiO₂-NPS. Furthermore, it significantly reduces the formation of root galls and the multiplication of nematodes (Ahamad and Siddiqui, 2021).

3.2.7.6 Polymer and mineral-based nematicides

The development of nanomaterial-containing nematicides has shown promising enhancements in the efficacy and application potential of bioavailable compounds for agricultural pest control. Bovine serum albumin nanoparticles have greatly enhanced the transport and toxicity of avermectin, resulting in noteworthy increases in larval stomach toxicity and distribution. This demonstrates their superior control efficiency compared to conventional formulations (Su *et al.*, 2020). Abamectin has been effectively encapsulated with plant virus nanoparticle resulting in improved soil mobility and regulated release. This encapsulation provides greater protection against root-knot nematodes in tomato seedlings (Cao *et al.*, 2015). The significance of nanoparticles in protecting photosensitive active components from UV degradation has been emphasised. Lecithin nanoparticles have been found to offer considerable UV shielding, hence maintaining the effectiveness of active chemicals (Chun and Feng, 2021). In addition, the process of enclosing avermectin in benzyl-modified chitosan oligosaccharide nano-capsules has not only enhanced its resistance to degradation by light, but has also resulted in a

higher level of effectiveness in killing nematodes. This is supported by the observation of a more extensive dispersion of the nano-capsules within the nematodes (L. Li *et al.*, 2023). The utilisation of avermectin-chitosan/poly- γ -glutamic acid nanoparticles has demonstrated a notable increase in nematocidal activity as a result of improved dispersion and absorption by nematodes (Liang *et al.*, 2018). Furthermore, the utilisation of nanocarriers for abamectin has exhibited enhanced movement, dispersion, and durability in soil, resulting in superior nematode management. This underscores the potential of nano-formulations in improving pesticide utilisation and advocating for sustainable agricultural methods (D. Zhang *et al.*, 2020).

3.2.8 Soil quality improvers

Soil quality improver materials are substances added to soil to enhance its physical, chemical, and biological properties, thereby improving plant growth and agricultural productivity.

3.2.8.1 Polymer-based soil quality improvers

The study undertaken by Kim *et al.* explored the application of a potassium acrylate-derived nanocomposite hydrogel as a superabsorbent polymer in soil, with adjustable swelling properties which positively affected tomato and cucumber growth (Kim *et al.*, 2022). A second investigation exploited a nanometric soil conditioner, which had a nano-networked structure, to reduce soil compactness, increase soil porosity and enhance water retention rate to maximise pepper yield (Cao *et al.*, 2023). The used nanomaterial also drastically changed the soil composition, enhancing the populations of earthworms, some microbial and fungal species.

The toxicity of a laponite polymer-based hydrogel in different dilution fractions was also investigated (Kim *et al.*, 2022). The results indicated the absence of cytotoxicity, as cell viability of at least 70% was observed in all dilution fractions. Moreover, comparable cellular attributes were observed in both the negative control and the hydrogel soil moisturiser, suggesting that the cells were robust and engaged in proliferation.

3.2.8.2 Calcium-based soil quality improvers

The potential of calcium-based nano-formulations to enhance soil performance and agricultural productivity has been demonstrated in two separate studies. The swelling capacities of carboxymethyl cellulose hydrogels were enhanced in comparison to unadulterated hydrogels upon the incorporation of nanocellulose crystals. As a consequence, the soil experienced a substantial enhancement in its capacity to retain water and emit carbon dioxide (Watcharamul *et al.*, 2022). Similarly, the integration of nano-gypsum and *P. taiwanensis* yielded substantial improvements in water consumption efficiency, soil properties, microbial communities, and nutrient control (Chaudhary *et al.*, 2021).

Due to the eco-compatibility and non-toxic characteristics shown, carboxymethyl cellulose/nanocellulose crystals composite hydrogels can be considered safe for application as soil amendments (Watcharamul *et al.*, 2022). It has additionally been demonstrated that neither the application of nano-gypsum had adverse effects (Chaudhary *et al.*, 2021).

3.2.8.3 Carbon-based soil quality improvers

In recent studies it has been demonstrated that the addition of carbon-based nanomaterials in specific concentrations to soil significantly improved soil quality and plant development, dramatically enhancing the biomass of shoots, as well as the concentrations of chlorophyll and nutrient uptake, without causing a significant change in root biomass (Nassaj-Bokharaei *et al.*, 2021; Nepal *et al.*, 2023). Additionally, multi-walled carbon nanotubes (MWCNTs) were found to be effective in promoting crop growth and reducing the bioavailability of residual herbicides, thereby mitigating phytotoxic effects (Yao *et al.*, 2021). Furthermore, it has been proven that urea-loaded cellulose greatly increased the soil ability to retain water and accelerated wheat germination, suggesting that it had potential as a slow-

release fertiliser (Y. Wang *et al.*, 2021). An additional publication documented how enzyme activities and soil respiration were enhanced by the application of graphene oxide (Hammerschmiedt *et al.*, 2023).

In terms of the toxicity of carbon-based nanomaterials, research has shown that although MWCNTs had no effect on the diversity of soil microbial communities overall, they significantly altered the functionality of the microbial ecosystem (Yao *et al.*, 2021).

3.2.8.4 Cerium-based soil quality improvers

Peng *et al.* explored cerium oxide nanoparticles use in soil improvement. Cerium oxide nanoparticles (CeO₂-NPs) demonstrated negligible impact on soil pH but significantly influenced both the soil redox potential and electrical conductivity, with a dose-dependent enhancement observed (Peng *et al.*, 2020). Furthermore, despite their low solubility, cerium nanoparticles remained in soil and altered the bioavailability of heavy metals.

3.2.8.5 Copper-based soil quality improvers

The use of copper-based nanomaterials in agriculture, such copper oxide nanoparticles (CuO-NPs), is increasing due to their capability to improve soil quality and, consequently, plants growth. Without significantly changing pH levels, these nanoparticles demonstrated to modify soil electrical conductivity, redox potential and the amount of soil organic carbon (Peng *et al.*, 2020; Xiaoxuan Wang *et al.*, 2021). Wheat seed priming using nanomaterials based on chitosan and copper promoted resistance to stress, by enhancing essential photosynthetic pigments and improving antioxidative activities (T. Farooq *et al.*, 2022). Although all the reported beneficial effects, a complex link between Cu-based nanoparticles concentration and plant growth was suggested by the possibility that larger concentrations could impede chloroplast activity (Nekoukhou *et al.*, 2023). Additionally, plants with lower concentrations of CuO-NPs had higher chlorophyll content and shoot biomass, which may indicate better photosynthetic efficiency and enhanced overall health. However, it was shown that the initial reduction of soil enzyme activities by CuO-NPs was temporary, since a recovery occurred after 30 days, and that Cu-based nanoparticles and soil biological processes interacted dynamically (Asadishad *et al.*, 2018). The combined results of these studies suggested that although Cu-based nano-materials can improve specific agronomic features, their effects on soil and plant systems were complicated and depending on dose, necessitating careful management to maximise benefits and minimise possible negative effects.

The eco-toxicological consequences of copper-based soil improvers in nanoforms were closely examined. The use of Cu-NPs was noteworthy because of its reduced negative effects on the environment if compared with traditional Cu-based agrochemicals. These nanoparticles were engineered to achieve their targeted benefits for improving soil quality with minimal ecological disruption (T. Farooq *et al.*, 2022). Changes in soil characteristics affected arsenic bioavailability, which could represent a concern to food safety (Xiaoxuan Wang *et al.*, 2021).

3.2.8.6 Iron-based soil quality improvers

It was demonstrated that iron-based nanomaterials, greatly improved soil water retention, maintaining moisture, enhancing germination rates and increasing chlorophyll content (Y. Wang *et al.*, 2021). In a study performed by Xu *et al.*, it was demonstrated that the application of nanomagnetite@dimercaptosuccinic acid affected soil gas emissions and nutrient cycling, particularly by raising CO₂ and nitrogen levels, with consequences for soil microbial ecology (J. Xu *et al.*, 2022). Evidently, there was a trade-off between microbial activity and plant productivity, as demonstrated by the use of zinc and iron oxide nanoparticles, which led to a decrease in microbial colony-forming units and nitrogen mineralization, thereby reducing radish yield (G. M. Shah *et al.*, 2022). In the same study, health risk assessment of iron-based soil improvers in nanoforms revealed that daily intake of metal and health risk index values for nanoparticles were within safe limits.

3.2.8.7 Magnesium and manganese soil quality improvers

Magnesium and manganese oxide (MgO and MnO) nanocomposites based on biochar proved to reduce sodium-related parameters in salt-stressed soils, while enriching essential nutrients and enhancing plant development and tolerance to salt (Farhangi-Abriz and Ghassemi-Golezani, 2021). Simultaneously, MgO-NPs showed significant Fe³⁺ and Cu²⁺ adsorption capability, affecting plant uptake and metal availability in treated soils with also effects on potassium, phosphate, and nitrogen levels (Eissa *et al.*, 2022).

3.2.8.8 Silica-based soil quality improvers

It has been demonstrated that nanometric silicate salts had major impact on soil pH, if compared to traditional Cu-based agrochemicals (Xiaoxuan Wang *et al.*, 2021). Moreover, the use of SiO₂-NPs, allowed the significant reduction of the bioavailability of dangerous metal such as Cu²⁺ and iron in soils, positively affecting the availability of essential nutrients such as nitrogen, phosphorus, and potassium (Eissa *et al.*, 2022).

3.2.8.9 Silver-based soil quality improvers

Silver nanoparticles (Ag-NPs) demonstrated to affect the biological and chemical features of soil. According to Asadishad *et al.*'s study (Asadishad *et al.*, 2018), Ag-NPs changed the structure and activity of soil microbes. Furthermore, they affected enzymatic activity proportionally to their concentration and showed a different microbial reaction if compared to the impact of Ag⁺.

3.2.8.10 Sulphur-based soil quality improvers

The use of nano-sulphur in soil improving has been investigated in the past years. According to published results, the use of nano-gypsum combined with *P. taiwanensis* greatly increased soil microbial populations, improving water and nutrient management, and having positive effects on crop productivity without being observably toxic (Chaudhary *et al.*, 2021). Furthermore, the combined application of low concentrations of nano-sulphur and graphene oxide increased soil respiration and enzymatic activity. On the other hand, nano-sulphur at higher doses became less beneficial (Hammerschmidt *et al.*, 2023).

3.2.8.11 Titanium-based soil quality improvers

Scientific evidences showed that plant length and biomass increased significantly when titanium dioxide nanoparticles (TiO₂-NPs) at a proper concentration were applied to soils (Arshad *et al.*, 2021), supporting the role of TiO₂-NPs in improving soil fertility and water retention. Moreover, a 30-day exposure study to TiO₂-NPs showed no apparent changes in the structure of the microbial community or soil enzyme activities, indicating a harmless effect on microbial ecology and soil biochemical processes at this nanoscale (Asadishad *et al.*, 2018).

However, at elevated concentrations of 750 mg kg⁻¹, TiO₂-NPs demonstrated to cause phytotoxicity, resulting in a 17–26% reduction in rice shoot biomass across a range of soil types as well as a significant decrease in shoot length and chlorophyll levels (Arshad *et al.*, 2021). The severity of impact was directly proportional to the clay content of the soils.

3.2.8.12 Zinc-based soil quality improvers

Multiple research efforts demonstrated that the application of zinc oxide nanoparticles (ZnO-NPs) affected a range of soil characteristics and the plant nutrients absorption. The effect of high concentrations of ZnO-NPs were mainly a considerable raise of the soil pH, due to hydroxide ion release during hydrolyzation, a significantly enhanced soil redox potential, with dose-dependent effects, and the increased soil electrical conductivity (Aziz, Shah and Rashid, 2019; Peng *et al.*, 2020). Additionally, ZnO-NPs effects on soil enzyme activities revealed that, even though initial exposure decreased enzyme function, a 30-day exposure period resulted in recovery and, in certain situations, even enhanced

activity (Asadishad *et al.*, 2018). ZnO-NPs had a good effect on plants uptake of zinc, significantly increasing the concentration of zinc in shoots. Under some nanoparticles treatments, there was a high translocation factor from roots to shoots, indicating better absorption efficiency. For heavy metals like copper and iron, ZnO-NPs shown a noteworthy adsorption capacity, which may lessen the metals availability in soil and be advantageous for soil remediation efforts (Eissa *et al.*, 2022). In a study performed by Knijnenburg *et al.*, it was demonstrated that zinc/alginate-based nanobeads had a notable release of zinc when compared to other types of beads and, for this reason, they could be used as slow-release soil fertilisers (Knijnenburg *et al.*, 2021).

On the other hand, it was found that applying ZnO-NPs to the soil decreased microbial biomass and colony-forming units, suggesting a possible negative effect on the health of soil microorganisms (G. M. Shah *et al.*, 2022). Furthermore, ZnO-NPs concentration and application technique significantly impacted soil micronutrient contents, negatively affecting soil microbial activities at higher concentrations (Verma *et al.*, 2022). According to health risk assessments, there were no major health concerns associated with zinc nanoparticles exposure, as indicated by the daily intake and health risk index, which were both within safe levels (G. M. Shah *et al.*, 2022). However, a study (Yuqi Wang *et al.*, 2021) reported that zinc-based nanoparticles influence the uptake of arsenic in plants, increasing the total concentration of arsenic in all the plant parts.

3.2.9 Virucides

Virucides are substances capable of inactivating or destroying viruses, often employed in disinfection procedures to minimise the spread of the virus. These agents target the viral structure, disrupting its ability to infect host cells and replicate.

3.2.9.1 Silver-based virucides

The majority of study concentrates on evaluating the efficacy of nanomaterials, specifically silver nanoparticles (Ag-NPs), in enhancing plant resistance against viral infections. For example, the application of Ag-NPs significantly enhanced the ability of tomatoes to resist the tobacco mosaic virus. This was achieved by reducing the levels of oxidative stress indicators, such as malondialdehyde and hydrogen peroxide, and thereby increasing the plant's antioxidative capacity and polyphenolic content (Al-Askar *et al.*, 2023). Similarly, Ag-NPs were shown to mitigate strawberry mild yellow edge virus in strawberries, maintaining bioactive constituents and altering the acidity and phenolic composition of infected fruit juice, highlighting a nuanced approach to viral defence (Shafie, R. M. and Abdelkader, H. S., 2023). Ag-NPs foliar treatment in squash plants demonstrated substantial efficacy in protecting against cucumber mosaic virus. This intervention resulted in delayed symptom onset, increased growth metrics, and elevated antioxidant enzyme activity (Abdelkhalek, El-Gendi, *et al.*, 2022). Faba beans that were exposed to Ag-NPs before being infected with bean yellow mosaic virus had enhanced resistance, as indicated by higher rates of photosynthesis, growth, and increased expression of stress-related genes (Abdelkhalek, Yassin, *et al.*, 2022). Ag-NPs exhibit phytotoxic effects that vary by crop species and life stage, dependent on nanomaterial-type, dosage, and growth conditions, affecting a wide range of physiological parameters during soil exposure (Adeel *et al.*, 2021).

3.2.9.2 Carbon-based virucides

Functionalized carbon quantum dots have demonstrated a significant reduction in virus accumulation in plants when used in combination with double-stranded RNA (dsRNA). This effect is observed through different application methods, such as infiltration, spraying, and root soaking. It is worth noting that the efficiency of virus suppression is enhanced when mixed dsRNA formulations are used (Xiang Xu *et al.*, 2023). In addition, the use of carbon nanotubes and fullerene treatments not only decreased the amount of viral coat protein transcripts and symptoms, but also increased the production of defence-related phytohormones and gene transcription associated with phytohormone biosynthesis. This provides a wider range of protection against viral infections in plants (Adeel *et al.*, 2021).

3.2.9.3 Chitosan-based virucides

Studies on chitosan-based virucides in nanoforms has demonstrated their strong antiviral effects, particularly through the use of targeted double-stranded RNA formulations and carbon dots nanoparticles. These solutions effectively reduce the accumulation of viruses in plants, with a dual effect on plant resistance to viruses: they decrease the severity of the disease and delay the onset of symptoms. Additionally, they promote an increase in total soluble carbohydrates, phenolic contents, and the upregulation of defence-related genes. This demonstrates that these formulations are a versatile and effective method for managing plant viruses (Abdelkhalek *et al.*, 2021; Xiang Xu *et al.*, 2023).

3.2.9.4 Iron-based virucides

Cai *et al.* investigated the absorption and dispersion of magnetite nanoparticles (Fe₃O₄-NPs) in plant tissues, discovering that these nanoparticles were taken in by leaf cells and spread throughout the plant via the vascular system. The Fe₃O₄-NPs retained their shape and considerably enhanced plant growth. Furthermore, they contributed to the improvement of plant disease resistance by increasing the activities of antioxidant enzymes and genes related to resistance, including salicylic acid-responsive genes. Likewise, Fe₃O₄-NPs demonstrated potent antiviral properties against tobacco mosaic virus by direct interaction with particles and stimulation of plant immunity (Cai *et al.*, 2020).

3.2.9.5 Polymer-based virucides

Chloroinconazide@alginate-based nanogel has demonstrated exceptional characteristics in enhancing the ability of liquids to spread and adhere to plant leaves, particularly those of the *N. benthamiana* species. This enables better utilisation and penetration of antiviral substances. The sustained release mechanism of this substance, along with its ability to activate reactive oxygen species, not only strengthens its antiviral effectiveness against tobacco mosaic virus at both the transcriptional and translational levels, but also improves plant development by releasing important nutrients such as Ca²⁺ and Mg²⁺ (Lv *et al.*, 2021). Furthermore, the use of star polycation-loaded calcium glycinate has demonstrated the ability to improve the calcium levels in tomato leaves, leading to higher photosynthetic rates and effective regulation of tomato plant growth. This, in turn, results in a notable decrease in the occurrence of diseases caused by tomato mosaic virus (S. Yan *et al.*, 2022). In addition, the use of polyetherimide-modified functionalized carboxylated single-walled carbon nanotubes (PSWNTs) to transport plasmid DNA into plant cells has demonstrated a highly promising method for managing viruses. This approach allows for a gradual and ongoing expression of the virus, resulting in significant protection against cucumber mosaic virus (Liu *et al.*, 2023).

The PSWNT-mediated plant virus cross-protection system demonstrates no short- or long-term toxicity, maintaining intact chloroplasts and similar photosynthetic quantum yields compared to control, with no oxidative stress induced in treated plants, evidencing its biocompatibility (Liu *et al.*, 2023).

3.2.10 Nanocarriers

A nanocarrier in the agricultural field is a nanoscale vehicle designed to transport and release active substances, such as fertilizers, plant protection product, or genetic material, directly to plants or soil.

3.2.10.1 Polymer-based nanocarriers

In a study by Zhang *et al.* (Y. Zhang *et al.*, 2023), the functionality of charged nanocarriers was demonstrated through their differential phloem loading and distribution in dicot (tomato) and monocot (wheat) plants, highlighting the superior transport efficiency of anionic nanocarriers compared to cationic counterparts and free gadolinium. Contrastingly, Shen *et al.* focused on poly(N-isopropylacrylamide)-based nanogels for smart pesticide delivery, significantly enhancing foliar wettability, photostability, and controlled-release properties (Shen *et al.*, 2022).

In a comprehensive study by Bonser *et al.* examining the impact of nanoparticle treatments on soybean growth, no significant differences were observed in germination rates, root growth rates, or plant

heights when comparing treatments (Bonser *et al.*, 2023). Despite variances in growth across days after planting and years, with soybeans showing slower maturity in 2022 and an overall shorter stature compared to 2021, these differences were not attributed to treatment effects. Similarly, the analysis revealed no significant influence of treatments on the hundred-seed weight, larval weights, disease incidence, or flowering, and no phytotoxic effects were noted post-nanoparticle spray application, indicating negligible impact of nanoparticle treatments on soybean developmental parameters.

3.2.10.2 Polysaccharide-based nanocarriers

In an investigation of organic molecule-based nanocarriers, the treatment of corn and rapeseeds with pesticides in conjunction with delivery systems revealed a notable augmentation in substance adhesion and grain penetration, without significant effect enhancement beyond 0.1% delivery system concentration. Polysaccharide arabinogalactan notably improved solubility and complex stability, especially for less soluble pesticides, compared to Na-carboxymethyl cellulose. Additionally, arabinogalactan's influence extended to lipid membranes, notably increasing lipid diffusion coefficients in liposomes, potentially indicating lipid extraction or membrane disruption. These findings underscore the critical role of polysaccharide-based delivery systems in agricultural applications (Selyutina, Khalikov and Polyakov, 2020).

3.2.10.3 Mineral-based nanocarriers

In recent studies, the mobility and interaction of bioavailable compound-based nanocarriers, specifically tobacco mild green mosaic virus nanoparticles (TMGMV-NPs), were investigated in various soil types, demonstrating enhanced functionality in agricultural applications (Venkateswaran *et al.*, 2023). TMGMV-NPs exhibited good mobility across different soil depths, with their recovery and mobility being influenced by soil characteristics, such as density and hydrophobicity. This suggests that the soil type and composition significantly affect the behaviour and effectiveness of nanomaterial-containing products. Furthermore, comparisons between TMGMV-NPs and other nanoparticles highlighted TMGMV's superior mobility, attributed to its physical properties and interactions with soil components. Concurrently, another study by Bonser *et al.* found no significant impact of nano-seed treatments on the growth rates, germination, or phytotoxicity in soybeans, indicating the nuanced effects of nanocarriers in plant agriculture (Bonser *et al.*, 2023). These findings underscore the importance of considering soil and environmental factors in the development and application of nanomaterials for enhanced agricultural productivity and sustainability.

3.2.10.4 Silica-based nanocarriers

In a study, it is reported the release behaviour of 1-naphthylacetic acid-silica nanospheres was significantly influenced by pH, temperature, and particle size (Ao *et al.*, 2013). The findings demonstrated that both acidic and alkaline conditions, as well as elevated temperatures, facilitated the cumulative release of active substances due to the amide linkages' higher decomposition rates. This was further substantiated by non-Fickian transport mechanisms, indicating an anomalous diffusion process. Additionally, varying the particle size of the nanospheres altered the release rates, with smaller particles showing quicker release, suggesting the importance of size selection for targeted delivery efficiency.

Parallely, research reported in on avermectin@mesoporous silica nanoparticles-ss-starch nanoparticles revealed negligible toxicity towards *Plutella xylostella* larvae, contrasting with the dose-dependent mortality observed with avermectin formulations (Liang *et al.*, 2020). Notably, the mortality rates and median lethal concentrations (LC₅₀) over time indicated a significantly enhanced efficacy of nanoparticle formulations, showcasing their superior sustained-release properties compared to traditional avermectin emulsifiable concentrates.

3.2.11 Arachnicides

Arachnicides are chemical agents or substances used specifically to kill or control arachnids, such as spiders and scorpions.

3.2.11.1 Chitosan-based arachnicides

Regarding chitosan-based nanomaterials exhibiting arachnicidal characteristics, only a single document has been identified. The arachnicidal activity of *Achillea millefolium L.* essential oil and its chitosan-encapsulated nanoparticles (A.EO@NPs) increased with higher A.EO concentrations, according to a study by Ahmadi *et al.* (Ahmadi *et al.*, 2018). Interestingly, the LC₅₀ values for A.EO@NPs were distinct from those of free A.EO, suggesting a difference in potency. The pH of the nanoparticle formulation notably influenced toxicity; higher pH values led to decreased toxicity, attributed to smaller nanoparticle sizes and reduced diffusion of A.EO from the nanoparticles. Moreover, A.EO@NPs formulations maintained their lethal effects over extended periods, in contrast to the rapid decline in mortality caused by free A.EO. The encapsulation also facilitated better diffusion through the mites' cuticles, enhancing contact lethality. The most notable toxicity and durability were shown by nanoparticles prepared at a pH of 5.5, indicating that the size of the nanoparticle played a crucial role in the effectiveness of arachnicidal drugs.

3.2.11.2 Copper-based arachnicides

Copper nanoparticles (Cu-NPs) effectively targeted all developmental stages of some arachnids, i.e., *P. oleivora*, *E. orientalis*, and *B. obovatus*, causing significant mortality and egg malformation while demonstrating a notable decrease in egg hatching rates across a gradient of concentrations, according to the study performed by Al-Azzazy *et al.*, which focused on copper-based nano-arachnicides (Al-Azzazy and Ghani, 2024). The predatory mites *A. swirskii* and *E. scutalis*, on the other hand, showed very little mortality, highlighting the selective toxicity of Cu-NPs.

3.2.11.3 Saponin-based arachnicides

It has been demonstrated that the application of curcumin nanoparticles and glycyrrhizic acid NPs significantly decreased the densities of *T. urticae* and the fertility of female mites. Comparable efficacy was observed at both high and low nanoparticle concentrations, indicating that lower concentrations may be advantageous. These findings, as reported in the research article published by Salama *et al.*, underscore the potential of both curcumin and glycyrrhizic acid ammonium salt nanoparticles in controlling mite proliferation (Salama *et al.*, 2022).

3.2.11.4 Polymer-based arachnicides

The use of organic polymers-based nano-arachnicides has been reported in recent years in the literature. According to Peng *et al.*'s study, cyflumetofen nano-capsules exhibited release behaviours that were dependent on pH and temperature (Peng *et al.*, 2019). Cyflumetofen was released more quickly at higher temperatures, although it released less quickly at higher pH values. Notably, the nano-capsules cumulative release rate exceeded that of the suspension on day 15, suggesting improved stability in water, especially at high pH levels. From day 4 onward, these nano-capsules demonstrated higher control efficacy against *P. citri*, revealing the advantages of prolonged release. A research compared the effects of nanoniosomes with nanoliposomes and conventional commercial formulations on mite survival (Teymouri *et al.*, 2022). Nanoniosomes achieved a 60% reduction in mite survival, outperforming both nanoliposomes and commercial alternatives. Additionally, sub-lethal concentrations of abamectin formulations significantly diminished mite fecundity and net reproductive rate, further showcasing the potential of nano-formulations in pest control. In the study performed by Yan *et al.*, the interaction between star polycations and the active molecule osthole was characterized by high affinity and strong binding, resulting in improved cytotoxicity of osthole against pests (Yan *et al.*, 2021). This complexation led to a more effective control of strawberry powdery mildew in organic agriculture settings, demonstrating the application of nano-delivery systems in enhancing pest and

disease management. Lastly, a research recently published highlighted the efficacy of 1,8-cineole in both its free and nano-emulsion forms (Ayllón-Gutiérrez *et al.*, 2023). The nano-emulsion, due to its improved chemical stability and sustained release, was nearly 2.5 times more effective than the free monoterpene form. The study underlined the enhanced bioactivity of nano-formulations against various pests, with *B. tabaci* showing the highest susceptibility.

Among the reported studies, just one of them explored the potential toxicity of the nanomaterial used. The research evaluates the safety and environmental impact of an osthole/star polycations nanocomplex used in strawberry production (Yan *et al.*, 2021). Enhanced plant uptake of osthole via star polycations did not elevate residual concentrations; instead, it facilitated a greater degradation rate, thereby reducing potential toxicity. Notably, the application of this complex exhibited negligible effects on strawberry fruit quality and posed no adverse effects on the egg hatch of the beneficial predator, *H. axyridis*. Furthermore, star polycations and related nanoparticles demonstrated minimal toxicity, affirming their applicability for sustainable agriculture.

3.2.11.5 Silver-based arachnicides

It has been demonstrated that silver nanoparticles (Ag-NPs) were able to significantly enhance arachnidicidal and ovicidal activities, with a notably higher specificity towards *O. afrasiaticus* compared to *N. barkeri* (Ghani *et al.*, 2024). Laboratory tests revealed an LC₅₀ value of 39.7 µg/mL for *O. afrasiaticus*, approximately 40 times more effective than for *N. barkeri* (LC₅₀ of 1587.9 µg/mL). Furthermore, Ag-NPs exhibited a potent ovicidal effect against *O. afrasiaticus* eggs, with an LC₅₀ of 67.8 µg/mL. Field applications at 216 µg/mL showed an 86.5% reduction in *O. afrasiaticus* populations and a modest 18.5% impact on *N. barkeri*, alongside suppressing 57.1% of egg hatching in *O. afrasiaticus*. These results revealed the potential of Ag-NPs as a selective and effective arachnidicide in pest management strategies.

In the same study, the nanomaterial toxicity was assessed by analysing residues of silver using ICP-OES spectrometry (Ghani *et al.*, 2024). The initial residues were found to be 1.83 µg/mL after application but decreased over time. The estimated daily intake was calculated to be 1.28 µg/kg/day, considering the highest residues obtained and the highest consumption rate worldwide. The hazard index averaged at 0.17, indicating that the level of residues was safe in terms of both acute and chronic toxicity.

3.2.12 Molluscicide

Molluscicides are chemical substances employed to manage or eradicate molluscs, including snails and slugs, that can cause harm as agricultural or horticultural pests.

3.2.12.1 Silver-based molluscicides

Recent studies have shown that silver nanoparticles (Ag-NPs) are more toxic and have greater functionality than their ionic counterparts in both marine and terrestrial ecosystems. A study by Zhang *et al.* demonstrated that oyster larvae, when exposed to Ag-NPs, accumulated approximately 6.7 times higher levels of silver compared to those exposed to silver nitrate (Zhang and Wang, 2023). It was found that a significant amount of the silver originated from particulate Ag. This study also emphasised the capacity of Ag-NPs to be traced within biological matrices via fluorescence imaging, uncovering substantial Ag⁺ absorption onto larval shells and distinct tissue distribution. Another study found that the exposure to Ag-NPs or titanium dioxide nanoparticles did not have a significant inhibitory effect on plant growth (Wu *et al.*, 2021). Nevertheless, it was discovered that the concentration of silver in plant shoots and its subsequent transfer to snails through the food chain were greater in exposures to Ag-NPs compared to silver ions (Ag⁺).

3.2.13 Nano-agrochemicals toxicity

3.2.13.1 Silver-based nano-agrochemicals

Silver-based NPs (Ag-NPs) are reported in 216 scientific articles (see Annex II, Section 8.2) mainly as bactericides, fungicides and fertilizers. Despite the large body of literature, as for all the other classes of nano-agrochemicals, no public document was identified to report the amount of Ag-NPs marketed and used in the EU or also worldwide. Nevertheless, Ag-NPs properties have attracted considerable attention as their potential toxicity and impact on biological systems require a comprehensive investigation. The research from Wang *et al.* demonstrated that exposure to Ag-NPs upregulates genes associated with the homologous recombination mechanism in plants and may alter epigenetic status, indicating a systemic response to genotoxicity (Wang *et al.*, 2019). Lahuta *et al.* conducted a study that provided additional evidence of the adverse impacts of biogenic Ag-NPs exposure on plant growth. The study specifically focused on wheat seedlings and observed that the exposure affected the development of the seedlings, as well as their cyclitol, toxicity, and carbohydrate profiles (Lahuta *et al.*, 2023). Similarly, synthetic and commercial Ag-NPs have been shown to reduce chlorophyll content and modify the biochemical profile in peanuts, affecting plant growth and fatty acid composition (Santos-Espinoza *et al.*, 2020).

The influence of nano-silver extends to the rhizosphere, significantly altering bacterial and fungal community diversity, with certain species showing increased abundance following exposure (Sillen *et al.*, 2020). Moreover, Ag-NPs negatively impact rice seedlings and rhizobacteria, potentially disrupting soil microbial populations and metabolites vital for plant growth and soil health (Mirzajani *et al.*, 2013). Ag-NPs exhibit lower phytotoxicity but higher fungitoxicity compared to Ag⁺ ions, posing a potential threat to soil microorganisms and overall ecological balance (Wang, Li and Shi, 2021). When Ag-NPs are used in conjunction with agricultural chemicals such as glyphosate, they synergistically inhibit wheat growth, induce oxidative stress, and alter microbial communities, further worsening their environmental impact (Feng *et al.*, 2021).

3.2.13.2 Zinc-based nano-agrochemicals

Similarly to Ag-NPs, zinc-based NPs uses are reported in 265 scientific articles (see Annex II, Section 8.2) and mainly used as fertiliser. Also in this case, no public information about their market use in the EU or worldwide is reported. Recent research has provided a clear understanding of the complex relationships between zinc oxide nanoparticles (ZnO-NPs) and different plant systems. These studies have revealed both positive and negative impacts, depending on the amount of nanoparticle dosage, the environmental conditions, and the presence of other compounds. The study conducted by Iftikhar *et al.* shows that the growth of wheat is affected by ZnO-NPs in a dose-dependent manner, with moderate doses enhancing growth attributes, while higher doses prove inhibitory (Iftikhar *et al.*, 2019). Interestingly, the application of gibberellic acid, improves some of the adverse effects, indicating a possible method to reduce stress caused by nanoparticles.

ZnO-NPs have demonstrated significant toxicity towards the fungal strain *F. solani*. This is evident from the comparatively low EC₅₀ values seen in both mycelial growth and spore germination assays, indicating an effective fungitoxic effect. In addition, the root elongation of tomato seeds was adversely affected by ZnO-NPs at all doses examined, indicating a substantial influence of ZnO-NPs on plant growth (Malandrakis *et al.*, 2021). In rice, ZnO-NPs exposure leads to significant zinc accumulation in various plant parts, with elevated atmospheric CO₂ levels modulating the impact on plant growth and nutrient content (W. Du *et al.*, 2022). Salicylic acid pretreatments have been found to be beneficial in enhancing the tolerance of *C. murale* to stress induced by ZnO-NPs (Taherbahrani, Zoufan and Zargar, 2021). This is achieved by regulating oxidative stress responses and metabolite production, pointing towards the potential for chemical treatments to counteract nanoparticle toxicity. Finally, this study investigates the ways by which ZnO nanoparticles are absorbed and transported into plants (Da Cruz *et al.*, 2019). It reveals that the size of the nanoparticles affects their dissolution and movement, which in turn has an impact on several physiological processes in plants.

3.2.13.3 Copper-based nano-agrochemicals

Copper-based NPs uses are reported in 184 scientific articles (see Annex II, Section 8.2) and mainly used as fertiliser, insecticide, arachnicide and nematocide, with no public information found on their market use in the EU or worldwide. While offering promising applications for enhancing plant growth and pest control, the potential risks of copper nanoparticles (Cu-NPs) and copper oxide nanoparticles (CuO-NPs) necessitate a balanced approach, emphasizing safe concentration thresholds, environmental sustainability, and the development of strategies to mitigate adverse effects.

In agriculture, CuO-NPs have demonstrated both beneficial and harmful impacts on plant growth and development. Studies on mung bean, *Arabidopsis*, maize, *C. melo*, spring barley, rice, and soybean have shown that low concentrations of CuO-NPs can sometimes enhance certain growth parameters or metabolic functions. For instance, CuO-NPs at 10 mg/L had no marked effect on mung bean root length and dry weight, with a slight increase in fresh weight observed, suggesting a potential role in enhancing certain growth parameters at lower concentrations (Karmous *et al.*, 2022). Conversely, higher concentrations were found to significantly inhibit growth, induce oxidative stress, and cause structural damage to plant cells and tissues (Nie *et al.*, 2020; C. Liu *et al.*, 2021; Jia *et al.*, 2022; Y. Chen *et al.*, 2022; Raza Khan *et al.*, 2023; Shah *et al.*, 2023).

CuO-NPs exhibit significant microbial toxicity, impacting the bacterial and fungus populations in soil. The exposure to CuO-NPs alters microbial community structures, enzyme activities, and metabolic profiles (Borymski *et al.*, 2023). In the Malandrakis *et al.* investigation, Cu-NPs exhibited a strong ability to inhibit both the mycelial growth and spore germination of *F. solani*. Additionally, they induced a significant increase in the levels of malondialdehyde and hydrogen peroxide in tomato plants, indicating high oxidative stress and damage to the cellular membrane (Malandrakis *et al.*, 2021).

Interestingly, certain strategies, such as the application of exogenous melatonin and hydrogen sulphide, have been shown to mitigate the phytotoxic effects of CuO-NPs. These treatments enhance plant tolerance to stress caused by CuO-NP by enhancing growth characteristics, antioxidant defences, and reducing metal accumulation (Jia *et al.*, 2022; Raza Khan *et al.*, 2023)

4 Survey on nano-formulated agrochemicals

4.1 Survey results

A total of 74 responses were obtained through the survey with an average of 7 minute and 49 seconds time to complete the 6 questions.

4.1.1 Question #1: Please select from the list below your occupation or the field in which your organisation is involved

Out of the 74 answers obtained, 59 researchers, 5 chemical manufacturers and 4 regulatory agency/consultant were reached while 6 responders replied "Other" being 5 representatives of non-governmental organization (NGO) and 1 from a civil society organization (CSO). In Figure 6 a graphical representation of the responders' profile is shown.

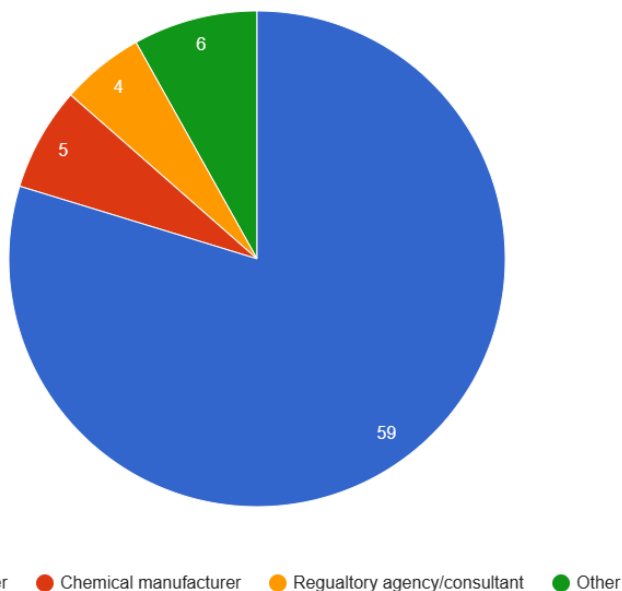


FIGURE 6 – QUESTION #1 ANSWERS REPRESENTATION

As expected, the survey, unfortunately, could not reach farmers. However, 7 responders answered the first question using the open-handed box whereby one also self-declared as additive manufacturer, considered as a chemical manufacturer, and another as a member of “Autoridade Tributária e Aduaneira” (considered as a regulatory agency/consultant). The last 5 responders did not indicate any pre-established category, thus their answer was reported in the “Other” category.

4.1.2 Question #2: Which areas are you presently exploring in connection with nanostructured agrochemicals?

For question #2, 74 answers were obtained with a wide variety of responses, also because no limit had been imposed to the number application areas that could be selected. The results are reported in the plot of Figure 7, where the height of the columns indicate the number of times that an application area was selected.

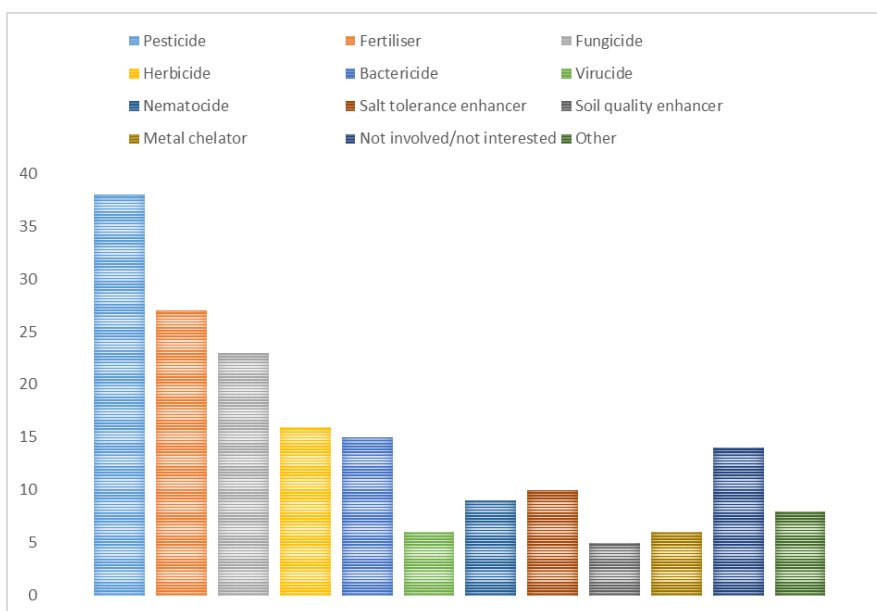


FIGURE 7 – QUESTION #2 ANSWERS REPRESENTATION

For the second question a variety of responses has been obtained, highlighting that different areas of interest are covered by the experts and users of nano-agrochemicals. Most of the responders (38) are involved in the usage of generic nano-pesticides, while the interest for nano-fertiliser products was expressed by 27 responders. In particular, nano-fungicide, nano-herbicide and nano-bactericide are explored by 23, 15 and 16 experts, respectively. The area of nano agrochemicals designated to enhance the tolerance to the abiotic stress is, interestingly, of relevance for 10 (salt tolerance enhancer) and 6 (metal chelator) responders, while only 5 responders indicated interest in nano-formulated soil quality enhancer. A minority of experts indicated interest in nematicide and virucide nano-agrochemicals, respectively 6 and 9. A non-negligible part of responders (14) indicated that are not currently involved or interested in the nano-agrochemical area. Among the 8 responders that used the open-handed answer box there was 2 interested in “Health and environmental impact”, 2 indicated interest in “Biostimulants”, 1 in “Biocide”, 1 in “Biosensor”, 1 in “Abiotic stress tolerance” and 1 in “Insecticide”.

4.1.3 Question #3: Can you indicate what is in our opinion the primary application(s) or area(s) of research or use for nanostructured agrochemicals?

For the third question, all responders indicated one preference. For this question a single answer was expected by each participant. Figure 8 shows the responses to question #3.

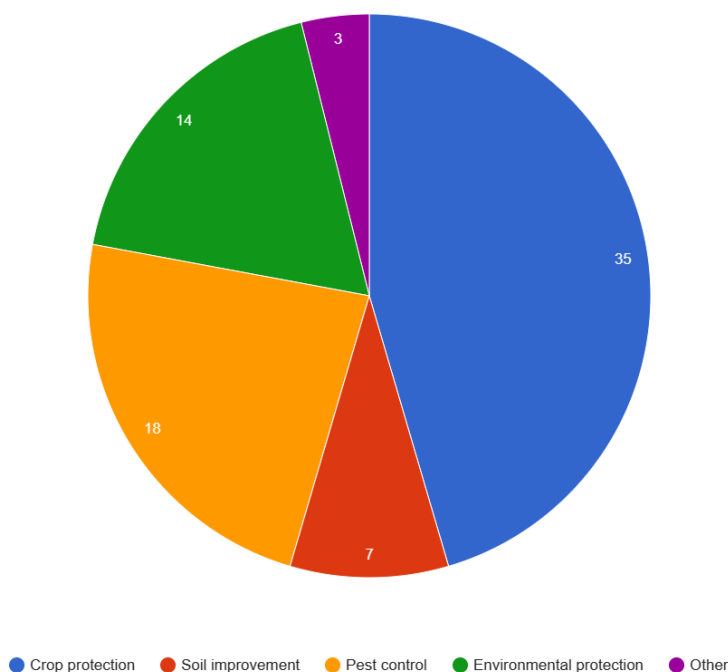


FIGURE 8 – QUESTION #3 ANSWERS REPRESENTATION

Interestingly, 35 survey participants indicated that the primary application of nano-agrochemicals is crop protection, while 18 of the responders are more incline to use nano-formulations for pest control. Only 14 attendees replied that the main application for nano-agrochemicals is the environmental protection, while 7 indicated the soil improvement as the primary application. Among the 3 responders that used the open-handed box, one replied “Nanostructured agro-chemicals should not be used in agriculture at all, their environmental risk is too high!”, another replied “nano nutrition” and the last one answered “Plant growth”. One participant replied, “all of the above”.

4.1.4 Question #4: Based on your knowledge or experience, what do you perceive as the key benefits or advantages of using nanostructured agrochemicals and related fields?

All participants responded to question #4. For this question, a maximum of three possible answers was expected, and results are depicted in Figure 9.

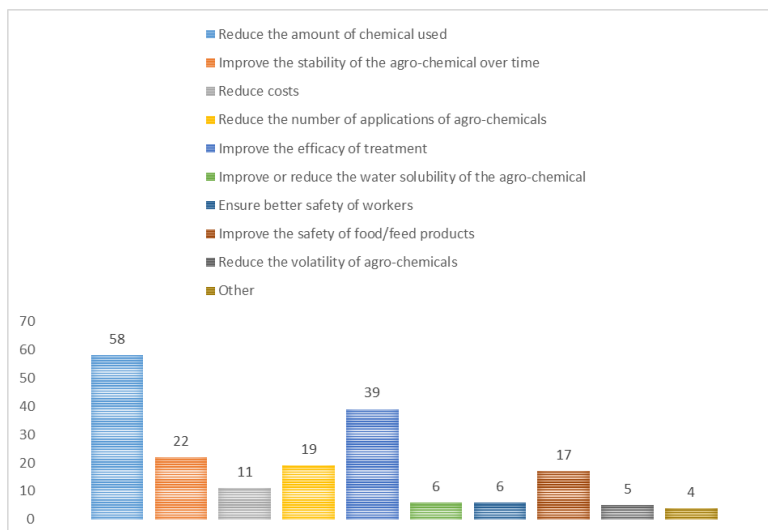


FIGURE 9 – QUESTION #4 ANSWERS REPRESENTATION

Most of the experts (58) identify the reduction of the amount of chemical used as one of the key benefits of the implementation of nano-agrochemicals. Also, the improvement of treatment efficacy is perceived as a clear advantage (39 responses). Similar results were obtained for the improvement of the stability of the chemicals over time (22 responses), the reduction of the number of application of agrochemicals (19) and the improved safety of food/feed products (17). Cost reduction is perceived as a minor advantage (11 answers), as well as the improvement of the water solubility of the nano-formulations (6 responses). Interestingly, only for 6 experts the enhanced safety for workers is seen as a key benefit from the usage of nano-agrochemicals. Lastly, only 5 experts indicated the reduced volatility of the chemicals as an advantage. The 4 participants that used the open-handed box to answer question reported as benefit the “Reduce N₂O emissions in atmosphere”, “Reduce environmental impacts”, “The danger of nano-chemicals is too high” and “None that merit the risks for workers, communities, environment”.

4.1.5 Question #5: Similarly, based on your knowledge or experience, what are the primary concerns or challenges associated with the use of nanostructured agrochemicals and related fields?

74 experts answered to question #5 with the possibility to select at most 3 different options. Figure 10 shows the results as a histogram.

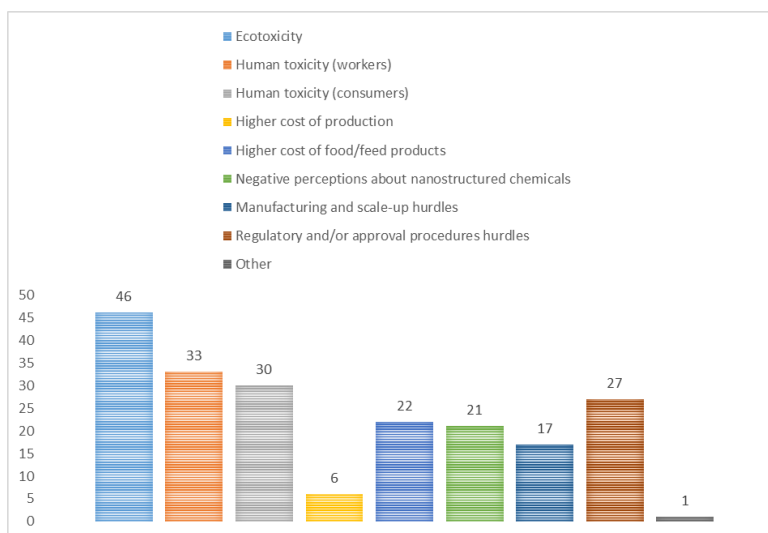


FIGURE 10 – QUESTION #5 ANSWER REPRESENTATION

Some interesting results have been obtained in the fifth question about the primary concerns or challenges associated with the use of nanostructured agrochemicals. Responders indicate that the ecotoxicity is a major concern with 46 preferences. Additionally, the regulatory and/or approval procedures are seen as difficulties and constitute a concern in the nano-agrochemical field (27 responses). Interestingly, the toxicity to humans of the nano-chemicals both for workers (33) and consumers (30) is indicated as a concern. Also, the higher cost of production compared to the traditional bulk chemicals is seen as a concern by 22 experts. Additionally, 21 participants responded that the negative perception about nanostructured chemicals is still a challenge for their utilization. The manufacturing and scale-up hurdles is seen as a challenge by 17 attendees, while only 6 responders indicated the higher price for food and feed treated with nano-agrochemicals as a primary concern. The only answer that was provided through the open-handed box was “general safety”, indicating again a concern about the toxicity of nanostructured compounds.

4.1.6 Question #6: In your opinion, what potential developments, advancements and uses do you foresee in the implementation of nanostructured agrochemicals and related fields?

Question #6 allowed experts to express their opinion on the potential developments, advancements, and uses envisioned in the implementation of nanostructured agrochemicals and related fields. A total of 38 experts contributed their insights in response to this open-ended query. In instances where comments contained private information, anonymization was achieved by inserting "(omissis)" within the original comments. It is important to note that no significant information was omitted during this process. The responses, with privacy-preserving edits, are comprehensively compiled in Table 11, providing a detailed overview of the anticipated future directions in this innovative area of agricultural science.

TABLE 11 – ORIGINAL ANSWERS TO QUESTION #6

Question number	Possible answers
#1	“In my opinion a greater usage of nano-fertiliser my become necessary to avoid crop losses due to the climate and ecological crisis (salt tolerance enhancer and fertilisers).”
#2	“Improve/assure the biocompatibility of nanomaterials”
#3	“Improve the safety of agro-chemicals”
#4	“The nanostructured agrochemicals have the potential to improve the effectiveness of disease and pest control while reducing the amount of active ingredients needed to achieve the same results. They could also be designed to release active ingredients in a controlled and targeted manner, potentially reducing environmental impact and improving safety.”
#5	“A possible shift from agro-chemicals based on the purely product/organism chemical interaction to a mixed physico-chemical product/organism interaction paradigm.”
#6	“Nanomaterials for the establishment of pesticide formulations. Environmental safety assessment. Production cost, evaluation standards and registration policies”
#7	“The use of nanostructured materials for the formulation of slow-release nitrogen fertilizers could contribute to reducing nitrogen losses in the environment both through volatilization, in the form of N ₂ O (the main greenhouse gas emitted by crops) and NH ₃ , and nitrogen losses through leaching.”
#8	“The lack of differentiation between Nano scale and Micro Scale. Reminder Nano scale is between 0 to 100 nm and over 100 nm we are in the micron scale, therefore we can ban the np up to 100nm and accept the particle up to 101 nm and higher.... the focus should be more on the chemical not really on size...”

#9	“Nanomaterials hold a Plethora of characteristics which benefits the formulation development.”
#10	“They can be used to control diseases of edible fruits and vegetables.”
#11	“Sustainable release systems and nano sensors”
#12	“Using any biosynthesized nanoparticles as agrochemical where these particles have multi advantages in agriculture field”
#13	“Nano-carrier-based formulations for pesticides are currently advancing rapidly. Readiness of regulatory agencies for their evaluation appears to be low.”
#14	“Sustainable improvement of preventive plant diseases as well as addressing to develop green curative plant diseases strategies”
#15	“Drug delivery for health and medical assistance. Control of antibiotic-resistant bacteria.”
#16	“The main potential is the reduction of the agrochemical amount. Nanoformulated agrochemicals are able to show similar efficacy and activity than classical agrochemicals by improvement of physicochemical properties. Lack of solubility and stability has affected to the required amount to observe efficacy in the current agrochemicals. Nowadays, this is a key point also affecting the resistance phenomena. However, nanostructured/nanoformulated compounds could help us to reduce the amount released to the soil and environment. Notwithstanding, it is really relevant to evaluate possible toxicity issues concerning this point due higher bioavailability properties.”
#17	“Better fertilizers, lower pollution, better ecological benefits.”
#18	“Sustainable way to crop production”
#19	“As plant biostimulants and microbial biostimulants”
#20	“Nanotechnology ensures higher crop production in climate-changing agriculture.”
#21	“Reduce the amount of chemical used”
#22	“Commercial production of nanostructured agrochemicals for sustainable agricultural development by reducing traditional agrochemicals in eco-friendly manner.”
#23	“The research on biomaterials for encapsulation, persistence in the plant, and movement through the food chain.”
#24	“Food and environment security”
#25	“Nanoformulations will open the development of new generation agrochemicals, such as those based on interfering RNA for specifically disrupt genes of plant pathogens and pest, with negligible effect on other organisms or the environment”
#26	“There could be some improvements in the efficacy of the nano-agro-chemicals but their application in real life will take longer. Meaning that their implementation in the field might go slowly.”
#27	“Nanostructured agro-chemicals hold great promise for revolutionizing the agricultural industry. With further advancements in this field, we can expect to see more targeted and efficient delivery of pesticides, herbicides, and fertilizers. This could lead to reduced environmental impact, lower usage of chemicals, and improved crop yields. Additionally, the development of nanostructured materials may also pave the way for innovative methods of soil remediation, plant disease management, and nutrient uptake enhancement. The potential uses of nanostructured agro-chemicals are vast, and ongoing research and development in this area are likely to uncover even more exciting possibilities in the near future.”

#28	"To produce functional food and embrace a more sustainable agriculture model"
#29	"Reducing traditional agrochemical quantity, harmonised test guidelines and regulation of this nano-agrochemicals"
#30	"Depends on the restrictions imposed, due to unpredictable regulatory environment it is hard to invest in a costly research"
#31	"I expect that the field will focus more on using natural products and biodegradable nanostructures to replace synthetic, persistent nanostructures - for instance, by replacing compounds like metals and plastics with i.e. nanoclays and lipids."
#32	"Agriculture with a sustainable approach"
#33	"Using nanostructured agrochemicals in developing nations will exacerbate the existing issue of chemical regulation in those regions. This is because nanostructured agrochemicals may present unique challenges in terms of monitoring and controlling their use, due to their small size and potential for increased reactivity. Additionally, the lack of infrastructure and resources for regulating these advanced products in developing nations could lead to misuse and environmental contamination. As such, careful consideration and collaboration between governments, industry, and regulatory bodies are essential to ensure the safe and responsible use of nanostructured agrochemicals in these regions."
#34	"Nano-enabled formulations are just now starting to enter into commerce in larger amounts and numbers- I see a lot more targeted approach to pesticide, herbicide and fungicide applications, especially for in-furrow applications, and importantly, more effort on behalf of companies to reduce potential ecological harm/maintain some degree of public acceptance. Encapsulation, delayed time delivery products etc will cut down on farm costs, amounts applied and reduced run-off after application are all developments coming about now."
#35	"implementation of nanostructured agro-chemicals should be more carefully examined due to their potential to cross biological membranes, even at the cell nucleus level. A cautious attitude should be prevailing before any implementation and commercial use."
#36	"We refer to the following publication to substantiate our above points: (<i>omissis</i>)"
#37	"The articles which are listed all agree on the necessary vigilance and transparency about the use of nanomaterials / nanoparticles / nano-objects in agro-chemicals. That's why we hope your report will consider this issue in depth, as there is, to date, no legal requirement on nanomaterials in agro-chemicals at the EU level (no specific labelling, no specific registration, no restriction, etc.). In France, the French nano-register shows that agriculture is the first sector in number of declarations. And the French national health and safety agency has had 20 pesticides tested by the French national metrology lab (LNE) to check whether they contain nanomaterials or not." (<i>omissis</i>)
#38	"Our current state of knowledge on nano-agrochemicals is not sufficient enough to guarantee that they are safe. Due to their properties, nano-materials are mobiles and can enter more easily the cells of non-target organisms, including humans. This also means that nanomaterials could also be present throughout the food chain, impacting directly the human health. Literature data point out that the environmental toxicity of pesticide active substances can be very different when they are formulated on a nanometric scale. However, There are currently no specific standardised methodologies for studying the toxicity of nano-pesticides and determining specific tolerance limits. In addition to the lack of knowledge regarding the toxicity of nanostructured agro-chemicals, there is today a lack of information about which substances (either active substances or co-formulants) is used in a nanometric form in pesticides / fertiliser. Plant protection products are not subject to any labelling requirements regarding the

nanomaterials. The lack of information about nanomaterials in pesticides is to be compared with the general lack of transparency on the formulation of pesticides. In summary, more data need to be generated on the environmental and health impact of nanomaterials. In the meantime, the precaution principle must be applied and the use of nanopesticide should be avoided. An obligation of labelling is needed to have more transparency regarding the use of these nanopesticides.” (*omissis*)

In summarizing the information collected from question #6, it becomes evident that the perspectives on the use and implications of nanostructured agrochemicals vary significantly among experts. Eight responses underscore the (eco)toxicological concerns associated with nano-agrochemicals, emphasizing the need for cautious advancement in this area. Yet, six experts see the shift towards nanostructured chemicals as a promising direction for reducing (eco)toxicity, suggesting a potential for environmental and safety improvements.

Five contributors express general apprehensions that are impeding the broader adoption of new nanotechnologies in agriculture. Among these concerns is the unpredictability of regulatory responses, which hampers investment in the research and development of nano-formulated compounds due to fears of regulatory hurdles. Three experts, however, view the adoption of nano-agrochemicals as an innovative approach to addressing the challenges posed by climate change and ecological emergencies, highlighting their potential to enhance plant productivity even under drought conditions.

Seven respondents anticipate advancements in the near future, suggesting optimism for the development and application of nano-agrochemicals. An equal number of participants express significant concerns regarding the widespread use of these materials, pointing out the necessity for a thorough assessment of their impact before large-scale introduction. One notable suggestion relates to the need of reforming the definition of nanoscale materials to focus on chemical properties rather than particle size alone, specifically recommending to focus on particles sized between 0 and 100 nm.

Additionally, there is anticipation for a shift in focus from synthetic nano-materials to more biodegradable options,, indicating a trend towards sustainability. It is essential to note, however, that within parts of the EU legislation, including the BPR, nanomaterials are typically defined as solid. This definition necessitates careful consideration when discussing alternatives like nano-emulsions or nano-droplets, which are liquid-based and may not fit traditional regulatory definitions of nanomaterials. Moreover, while the biodegradability of nano-materials such as nano-clays might suggest a sustainable alternative, it is important to recognize that not all components of clays are biodegradable. For instance, clay minerals such as kaolinite, consist of hydrous aluminium phyllosilicates which may not degrade biologically, thereby complicating their classification as fully sustainable materials. In addition, the presence of impurities such as iron oxide can also influence the environmental impact and colour of natural clays, adding further complexity to their use in agriculture. This variety of perspectives underscores the complexity of integrating nanostructured agrochemicals into agricultural practices. It highlights both the potential benefits and the challenges that need to be addressed, especially in terms of regulatory compliance and environmental sustainability. Such considerations are crucial for ensuring that the development of nano-agrochemicals aligns with both current regulations and future sustainability goals..

Respondents also indicated the following documents and public resources that were collected after the completion of the ELS and are here disclosed for transparency:

- [Study proposal](#) for EUON: information on nano-enabled pesticides & fertilizers proposed by the NGO AVICENN in partnership with other European NGOs (CIEL, ClientEarth, EEB and HEAL).
- Nanomaterials and agriculture – [Bibliography](#) provided by AVICENN.

- Sustainability Claims of Nanoenabled Pesticides Require a More Thorough Evaluation, Niderstigt T, Environ. Sci. Technol., 58, 5: 2163–2165, January 2024, <https://doi.org/10.1021/acs.est.3c10207>

5 Discussion

Drawing upon the findings of the ELS and the discussion of relevant documents as depicted in the previous chapter, the elements describing the adequacy of current practices and regulatory frameworks to ensure the identification and safety of nanomaterial-containing substances and products are critically evaluated. This includes an assessment of legislation such as the Biocidal Products Regulation (EU) No 528/2012, Plant Protection Products Regulation (EC) No 1107/2009, and Fertilising Products Regulation (EU) No 2019/1009. Additionally, recommendations are elaborated to increase the quantity and quality of information on both conventional and nano-enhanced products, from technical and legal perspectives. This encompasses the consideration of nano-sensors for detecting nanomaterials and nano-enabled delivery systems for nutrients, among other innovative approaches.

Interestingly, chapter 3 allowed to obtain insights into the diversity of nano-agrochemicals used across various applications, identifying different types of nanocarriers associated with bioactive chemicals loaded. The discussion below focus on evaluations and discussions that cross all these classes, highlighting the general challenges and opportunities presented by these nano-agrochemicals in enhancing product efficacy, safety, and environmental compatibility.

5.1 Evaluation and discussion of the adequacy of the current practices and regulatory frameworks

This section critically evaluates and discusses key aspects related to current practices and regulatory frameworks, based on three elements, as described above: a thorough ELS, meticulous document analysis, and insights from a survey. The discussion covers a wide range of important aspects, such as analytical techniques, quantification methods, and the available information on toxicity and ecotoxicity. Every aspect is carefully examined to determine its suitability and pinpoint areas that need improvement in order to align with the changing landscape of nanomaterial applications in agricultural products.

5.1.1 Analytical techniques

The ELS enabled the identification of various crucial analytical techniques that are essential for characterising and analysing nanomaterials within the context of this report. Each technique offers a distinct contribution to the comprehension of nanomaterial properties and behaviours, which is crucial for regulatory assessment and safety evaluation. It is important to mention that characterization parameters of nano-agrochemicals most commonly taken into account are shape, particle size distribution, crystallinity, specific surface area, surface treatment, chemical composition, zeta potential, and porosity. Notably, parameters such as particle size distribution, shape and specific surface area are directly linked to the efficacy and safety of nano-agrochemicals, potentially aligning with regulatory requirements for health and environmental impact assessments. Crystallinity, agglomeration potential and chemical composition are crucial for understanding the stability and degradation behaviour of these materials, pertinent to their lifecycle analysis and regulatory compliance. Additionally, surface treatment and zeta potential provide insights into the interaction of nanomaterials with biological systems, relevant for toxicity and ecotoxicity. These characterization parameters, along with their implications for regulatory standards, i.e. for the identification of analytical procedures to characterize, identify, and quantify nano-agrochemicals, will be discussed in the subsequent sections. Analytical techniques useful to tackle these challenges for identification, characterization and quantification of nano-agrochemicals resulting from the ELS, are listed as follows:

- Transmission Electron Microscopy (TEM) provides high-resolution imaging, allowing the precise visualisation of the morphology and crystallographic and porosity structure of nanomaterials. TEM

is useful for assessing the size, size distribution, shape, and compositional heterogeneity of nanoparticles.

- Scanning Electron Microscopy (SEM) offers insights into the surface topography and composition of materials, enabling the analysis of nanomaterial surface properties and the determination of size distributions in samples.
- Dynamic Light Scattering (DLS) is used to determine the size distribution of particles within a liquid, providing information on the hydrodynamic diameter of nanoparticles. This measurement is essential for comprehending their behaviour in biological systems. It is important to note that the hydrodynamic diameter can differ significantly from the actual or effective diameter of the particles. This discrepancy arises because the hydrodynamic diameter includes the particle along with its surrounding solvent, reflecting the dynamic interaction between the nanomaterial and the fluid. As such, the hydrodynamic diameter is not a fixed property but varies based on how the nanomaterial interacts with its surrounding environment. Furthermore, the hydrodynamic diameter is the diameter of the moving particle, so for moving aggregates and agglomerates the measurement will provide information on the whole aggregate / agglomerate and not on constituent particles with these.
- Atomic Force Microscopy (AFM) provides high-resolution imaging at the nanoscale and enables the quantification of the force exerted between the probe and the sample. This allows for the examination of surface roughness, morphology, and mechanical characteristics of nanomaterials.
- The Brunauer-Emmett-Teller (BET) is primarily utilized to measure the specific surface area of powder materials, which is crucial for understanding the reactivity and catalytic activity of nanoparticles.
- X-ray Diffraction (XRD) is crucial in determining the crystalline structure of nanomaterials, allowing for the identification of phase composition and crystallite size, which are vital for material characterization.
- Infrared (IR) or Near-Infrared (NIR) spectroscopy enables the chemical analysis of nanomaterials, facilitating the detection of functional groups and the evaluation of chemical durability and interactions with biological substances.
- Energy Dispersive X-ray Spectroscopy (EDX) is an analytical technique used to characterize heterogeneous compounds by determining their elemental composition. It is often used in conjunction with SEM and is particularly useful for identifying heavy metals and other high atomic number elements in complex mixtures.
- Nano-sensors refer to devices capable of detecting and measuring signals derived from nanoscale particles and substances, rather than being nanosized sensors themselves. The existing literature reveals a growing interest in the development of these technologies, although direct applications to nano-agrochemicals remain relatively sparse. Currently, the technology is in its nascent stages with regard to regulatory applications, indicating that while promising, nano-sensors require further development and validation before they can be widely implemented for monitoring and regulatory purposes in the context of nano-agrochemicals.
- Other analytical techniques for identification, characterization and quantification in biological matrices. Several techniques, such as sp-ICP-MS (single-particle inductively coupled mass spectrometry), PTA (particle tracking analysis), FFF, AF4, filtration techniques, fluorescence-tag microscopy, Raman spectroscopy, TOF-SIMS, CLS, TGA and analytical centrifugation, can be

employed to trace and observe nanomaterials within biological systems, offering crucial insights into the dispersion, agglomeration and aggregation state, and potential uptake of nanoparticles. Additionally, combined method such as AF4+DLS+MAL are a valuable alternative to nanoparticles identification. However, those methods are not widely used in the relevant documents retrieved with this ELS, resulting in a scarcity of information regarding their performance.

- Hybrid or combination approaches. The analysis of nanomaterials, especially in the context of nano-agrochemicals, necessitates a multifaceted approach due to the complex nature of these substances. Recognizing that no single analytical technique can provide a comprehensive understanding of nanomaterial characteristics, the adoption of hybrid or combination approaches becomes indispensable. These approaches leverage the strengths of various analytical methods to offer a more complete picture of nanomaterial properties, behaviours, and interactions.

These techniques collectively constitute the fundamental basis of nanomaterial analysis, with each technique providing unique benefits for thoroughly comprehending and assessing the characteristics and potential consequences of nanomaterials in agricultural applications.

5.1.2 Quantification of NPs and their active ingredients

Although section 5.1.1 presents a variety of advanced analytical techniques, there is still a significant difficulty in accurately measuring the amount of nanoparticles in particular conditions. In fact, while these methods are adept at identifying the presence, location, and detailed characterization of nanoparticles in upstream products, they have inherent limitations at low concentrations of nanoparticles or if the nanoparticles are embedded in complex matrices, which is often the case when analysing downstream samples. This constraint arises also from the fact that techniques such as TEM, SEM, DLS, AFM, BET, XRD, IR/NIR spectroscopy, and fluorescence-tag microscopy mainly offer qualitative or semi-quantitative data, prioritising physical and chemical characteristics rather than accurate concentration measurements. Particularly for quantifying downstream samples, the challenge often relates to determining the amount of active substances and their loading on the nanoparticles, rather than the nanoparticles themselves.

In the direction of enhancing this framework, [ISO/TC 229](#) standards provides information for the regulatory and safety evaluation of nanotechnologies in agriculture. Standards like ISO/TS 21361:2019, which delineates methods to quantify air concentrations of carbon black and amorphous silica in the nanoparticle size range in manufacturing environments, are crucial for environmental safety assessments. This standard aids in understanding the dispersion and potential respiratory exposure to nanoparticles, relevant for monitoring nano-agrochemicals in the field. Furthermore, ISO standards such as ISO/TS 12025:2021, which quantifies nano-object release from powders, and ISO/TR 19716:2016, focusing on the characterization of cellulose nanocrystals, support the identification, quality control, and compliance of nano-agrochemicals with established safety norms.

The challenge of regularly determining nanoparticle doses is significant, particularly considering the established link between dose levels and the likelihood of toxicity and ecotoxicity. Scientific research consistently emphasises that the effects of nanoparticles on biological systems are influenced by the amount of exposure, with varying concentrations resulting in distinct biological responses. The dose-response relationship is essential for evaluating the safety and environmental consequences of nanomaterials. It requires precise and dependable quantification techniques to effectively anticipate and minimise potential risks.

Given the lack of accurate methods for measuring nanoparticle levels and established standards for assessing their concentrations in upstream products or after their use in downstream samples, it is crucial for regulatory bodies to promote the disclosure of the exact composition of upstream products containing nanomaterials but also foresee the potential exposure scenarios for downstream uses.

Moreover, it is essential to provide explicit instructions on the utilisation of nano-enabled chemicals, i.e. chemical substances or materials that have been engineered at the nanometer scale to achieve distinct properties or functionalities not observable at larger scales, encompassing comprehensive guidelines for farmers and other individuals who utilise these substances. This approach not only addresses the current limitations in analytical methodologies but also improves the safety and environmental management of nanotechnology applications in agriculture.

5.1.3 *Sampling and isolation of NPs*

The process of sampling and isolating nanoparticles from complex matrices such as soils, water, and plant tissues present its own set of challenges within the realm of nanomaterial research and regulatory assessment. The inherent challenge of cells readily absorbing nanoparticles and incorporating them into their structures significantly complicates the process of isolating and analysing these particles. This issue is further compounded by the difficulties associated with detecting nanoparticles at low concentrations within complex environmental matrices, where the presence of various substances can obscure or interfere with the nanoparticles' response signals, making it more challenging to identify and quantify the nanoparticles reliably. Moreover, a crucial distinction must be acknowledged between sampling and isolation of nanoparticles in upstream products, such as raw materials or intermediates, and downstream samples, which include final products or environmental specimens. In the case of downstream samples, the task becomes notably more complicated due to the potential alterations and interactions that nanoparticles undergo in real-world conditions. Furthermore, the structure and chemical properties of nanoparticles may undergo modifications over time, particularly when deployed in real agricultural scenarios. The possibility of having ever-changing characteristics of nanoparticles introduce an additional level of complexity and would alter the ideal conditions for precise sampling, as the post-application transformations affect the detection and assessment of these nanoparticles in various matrices.

To address the temporal variability, it is essential to adopt a strategic sampling approach that considers the possible alteration of nanoparticles in environmental and biological contexts. Nevertheless, the lack of explicit and universally applicable operational protocols for sampling various categories of nanoparticles, as mentioned in the preceding section, intensifies the difficulty. The accurate sampling and isolation of different types of nanoparticles and matrices may necessitate distinct approaches, thereby adding complexity to the development of standardised methods.

Given these challenges, promoting transparency from manufacturers and producers becomes even more crucial. As mentioned earlier in the discussion on quantification, the development of standardized techniques for sampling and separating nanoparticles has shown promising progress. Despite these advancements, it remains essential for regulatory authorities to ensure that manufacturers provide detailed and accurate data on the composition and characteristics of nanomaterials used in their products (upstream characterization). This requirement should extend to information about the performance of these nanomaterials in different environmental conditions (downstream characterization). This should encompass information regarding the anticipated alterations in the structure of nanoparticles as time progresses, as well as their interactions with environmental and biological systems. Such measures are crucial, not because of the absence of standardized techniques, but to complement these emerging methods by guaranteeing transparency and enhancing the reliability of nanomaterial assessments in regulatory processes.

5.1.4 *Influence of particle size and shape*

The size and shape of nanoparticles used in agrochemicals is underscored by the pivotal role in defining nanoforms and framing regulatory guidelines. This relationship between nano-agrochemicals' physical attributes and their regulatory considerations stems from the profound influence of size and shape on efficacy, toxicity, and ecotoxicity as evidenced by many documents found in the ELS. The criticality of these factors is further highlighted by the exclusion of high number of documents from consideration,

owing to the nano-agrochemicals' divergence to conform to the nanosized material definition—being sized over 100 nm—or their application falling outside the tender's scope, such as in pharmacology or medical devices.

The ELS provided several insights delineating the impact of size and shape. Among these, research on carbon quantum dots as carriers for insoluble pesticides stands out, demonstrating that the solubility, absorption, and translocation of active chemicals within plants are intricately size-dependent. A study on wheat illuminated the beneficial effects of hematite nanoparticles, within the 20-40 nm size range, on plant biomass, chlorophyll content, and water status, showcasing their potential in ameliorating iron deficiency without adverse effects. Moreover, the antifungal prowess of reduced graphene oxide-copper oxide nanoparticles against *F. oxysporum* was shown to escalate with a decrease in size, underscoring a size-dependent mechanism. This size dependency is echoed in the antifungal activity of sulphur-based nanoparticles where the inhibition of fungi growth by nanoparticles smaller than 35 nm hints at the disruption of fungal cell wall structures, a proof to the critical role of nanoparticle size. In the domain of insecticide efficacy, nano-pesticides like avermectin-propylene glycol alginate formulations emerge as superior to conventional solutions against pests such as *Plutella xylostella*, thanks to their reduced size and increased surface area. These nanocarriers not only enhance the potency of active ingredients but also facilitate their distribution within plant systems, promoting growth under challenging conditions. Interestingly, the hydrodynamic sizes of Cu-NPs were found to inversely correlate with ecotoxicity in the Gram-negative strain *P. fluorescens*, although such a systematic size dependency was not observed in the Gram-positive strain *B. subtilis*. The antibacterial activity of ZnO-NPs is notably attributed to their small size and high surface area-to-volume ratio, highlighting the antibacterial potential inherent in nanoparticle size. This attribute also influences the release rates of nano-spheres, with smaller particles exhibiting quicker release, thereby underscoring the importance of size in achieving targeted delivery efficiency. The toxicity and durability of nanoparticles further indicate the significance of nanoparticle size in the efficacy of arachnidicidal drugs, affecting their dissolution and movement and, consequently, various plant physiological processes.

Given the preponderance of studies primarily focusing on the efficacy of nano-agrochemicals in evaluating size and shape, it becomes relevant for regulatory frameworks to integrate these factors into their evaluation processes. The evidence from the literature unequivocally highlights the critical role of size, shape and surface in determining the performance and environmental impact of nano-agrochemicals. As such, these findings advocate for a regulatory paradigm that not only acknowledges but also systematically incorporates the dimensions of size and shape into the assessment and approval of nano-agrochemicals, ensuring a nuanced understanding and management of their applications and implications.

5.1.5 Relationship between dose of NPs and dose of active substance

The relationship between the dose and its consequent effects - encompassing efficacy, toxicity, and ecotoxicity - is pivotal, as underscored by evidence from the ELS. The details of this dose-dependency reveal that the impact of nano-agrochemicals is not linear, with several studies highlighting the nuanced behaviour of nanoparticles in varying concentrations. For instance, research on the effects of zinc oxide nanoparticles (ZnO-NPs) on microalgae demonstrated a concentration-dependent toxicity. At lower doses, the impact on microalgae was minimal, but at higher concentrations, significant growth suppression and structural changes in the organisms were observed. This phenomenon suggests potential benefits in using lower doses of certain nanoparticles, such as silver nanoparticles (Ag-NPs), over higher doses of more conventional metal nanoparticles like iron and zinc, which have exhibited toxicity. Similarly, silica nanoparticles (SiO₂-NPs) at specific levels can enhance a plant's stress resilience, whereas higher concentrations lead to negative outcomes such as decreased shoot weight, increased lipid peroxidation, and enhanced catalase activity, indicating possible toxicity and stress. The case of chitosan with poly(methacrylic acid) (CS-PMAA) further illustrates the dose-dependent nature of nano-agrochemicals. While lower doses may promote growth and offer benefits, elevated

concentrations have been found to inhibit root growth, increase seedling mortality, and cause DNA damage, underscoring the critical need for dose optimisation in the use of CS-PMAA nano-fertilisers to ensure their safety and effectiveness in agricultural applications. It is critical to acknowledge that the efficacy and safety of nanoparticle usage are highly contingent upon the species involved. The decision to prefer one type of nanoparticle over another is profoundly influenced by the specific environmental context, the mode of action (MoA) of the nanoparticles, and the organisms in question. This necessitates a comprehensive understanding of both target and non-target organisms to tailor nanoparticle use effectively. The selection of nanoparticles for a particular application should therefore be guided by a thorough evaluation of their environmental impacts, considering the MoA and the susceptibility of both target and non-target species to ensure minimized ecological disruption.

The implications of these findings for regulatory frameworks are profound because the endpoint of agrochemical efficacy is different from the endpoint related to toxicity or ecotoxicity. Thus, recognising and accurately gauging the dose-relationship as crucial, yet it presents numerous challenges to evaluate. These challenges are not solely attributed to the variety in carriers but also to the active substances they deliver. Additionally, distinguishing the dose effects of the nanocarrier from those of the active substance adds a layer of complexity, especially when considering the differing persistence of nanoparticles and active substances in the environment over time. For instance, a nanoparticle may degrade, while the active substance remains, leading to divergent environmental impacts. Given these complexities, it is clear that a one-size-fits-all approach to measuring dose relationships cannot be a reliable method. Instead, tailored dose-relationship tests, potentially using a broad spectrum of model organisms and systems are essential for accurately assessing the multifaceted impacts of nano-agrochemicals. For instance, an approach should not be limited to aquatic models like zebrafish, which, while valuable for understanding MoA and omics studies, represent just one aspect of environmental impact. It is imperative to also incorporate soil-based models and more complex ecosystem simulations, such as microcosms or macrocosms, that include various interacting organisms. Soil-dwelling organisms like earthworms, nematodes, and soil microorganisms, as well as plant models, are crucial for evaluating the terrestrial impacts of nano-agrochemicals. These models can provide insight into bioaccumulation, translocation, and potential toxicity in soil ecosystems, offering a more comprehensive understanding of nano-agrochemical behaviour and their effects on different environmental compartments. Such specific but diversified investigations are vital to developing a nuanced understanding of nano-agrochemicals' dose-dependent behaviours, ensuring that regulatory frameworks can effectively manage these substances to harness their benefits while mitigating potential risks.

5.1.6 *Stability, bioaccumulation and controlled release of active substances*

In the regulatory framework for agrochemicals, the stability, bioaccumulation, and controlled release are crucial factors that can significantly influence both the efficacy and the toxicity or ecotoxicity behaviour of these chemicals. These attributes determine how agrochemicals interact with their environment, plants, and target pests, highlighting the importance of understanding and managing them to ensure safe and effective use.

The stability of nanoparticles (NPs) plays a pivotal role in enhancing the properties of active substances. For example, the development of prochloraz nano-capsules incorporating Fe₃O₄-NPs has shown to enhance the delivery and photostability of fungicides, leading to increased adhesion. Similarly, NPs can maintain stability across various water conditions or pH levels, thereby improving the agrochemical's effectiveness. The case of the avermectin nano-delivery system is notable, where even in lower quantities, it provides much greater insecticidal effectiveness against *Mythimna separata* compared to conventional avermectin suspensions. Furthermore, the stability afforded by nano-capsules facilitates the better penetration of genetic materials, as seen in advancements in RNA-based nano-formulations for targeted pest control.

The ability to control the release of active substances is a key objective in encapsulating agrochemicals. This is achieved through various means, such as polymer-based fertilisers that allow for the gradual release of nutrients through Fickian diffusion in polymer-based hydrogels, aligning with the European Fertilising Products Regulation (EU 2019/1009) regulation for slow-release fertilisers and minimising environmental impact. Zinc/alginate-based nanobeads have been highlighted for their effective slow-release properties as soil fertilisers. Similarly, terpenoid-based insecticides with avermectin nano-delivery systems demonstrate exceptional storage stability and pH-sensitive release mechanisms, offering regulated and prolonged release. However, the need for careful dosage control is underscored due to potential negative effects of high concentrations of carriers like chitosan nanoparticles (CSNPs). Controlled release mechanisms also contribute to reduced toxicity by limiting environmental exposure.

Bioaccumulation does not always correlate with toxicity or ecotoxicity. For instance, TiO₂-NPs, despite evidence of accumulation, did not exhibit negative consequences (Pérez-Zavala *et al.*, 2022). In contrast, MgO-NPs treatment significantly enhanced magnesium accumulation in plant tissues, promoting nutrient distribution. However, the application of Mn-NPs showed variable effects on wheat growth without significantly impacting yield, but altered nutrient distribution negatively (Dimkpa *et al.*, 2018). Conversely, CuO-NPs and Ag-NPs have shown to cause sensible bioaccumulation with adverse effects on plant physiology and aquatic organisms, respectively, highlighting potential ecological concerns related to their use (Huang *et al.*, 2019).

5.1.7 Interaction with ecosystems

Understanding the interaction of nano-enabled chemicals with biological ecosystems is fundamental to elucidating their mechanisms, efficacy, and ecotoxicity behaviours. These interactions are complex, as evidenced by varying results across different studies, underscoring the importance of fine-tuning nanoparticle concentrations and formulations to enhance the benefits of nanomaterial-containing fertilisers while mitigating potential toxicity.

Research has demonstrated that maize plants exposed to iron nanoparticles, including Fe and Fe₃O₄-NPs, showed a significant increase in leaf iron content, photosynthesis rate and biomass, without inducing oxidative stress. This indicates a beneficial impact on plant development through enhanced photosynthetic efficiency. However, the application of iron-based fungicide nanomaterials in agriculture reveals a nuanced picture, with both beneficial and toxic effects, on plants and animals. Despite their effectiveness in preventing plant diseases, these nanomaterials have exhibited complex toxicity profiles due to their interaction with biological systems. For instance, the positive surface charges on iron oxide nanoparticles can disrupt cell membrane permeability and facilitate non-specific interactions, leading to increased intracellular accumulation with genotoxic and cytotoxic consequences.

Further studies have shown that the initial reduction in soil enzyme activities caused by CuO-NPs is temporary, with a recovery observed after several days. This dynamic interaction between Cu-based nanoparticles and soil biological processes indicates that, while these materials can enhance specific agronomic traits, their overall impact on soil and plant systems is intricate and dose-dependent. Such findings highlight the necessity of careful management to optimise the benefits of Cu-based nanomaterials and minimise adverse effects.

Moreover, the soil type and composition play a crucial role in determining the behaviour and effectiveness of nanomaterial-containing products. For example, comparisons between tobacco mild green mosaic virus nanoparticles (TMGMV-NPs) and other nanoparticles have shown TMGMV's superior mobility, which can be attributed to its physical properties and interactions with soil components. This further emphasises the significance of considering environmental variables when assessing the potential of nanomaterials in agriculture.

5.1.8 Considerations on toxicity and ecotoxicity

The discussion on the toxicity and ecotoxicity of nano-enabled chemicals with agrochemicals is complex, showing a range of effects influenced by several factors. This complexity is further compounded by the subjective nature of determining the purpose and functionality of nano-agrochemicals, making the management, and understanding of their application in agriculture critically important.

For instance, iron-based fungicide nanomaterials exemplify the dual nature of nanocarriers, presenting both beneficial and toxic effects on plants and animals. While these materials can enhance agricultural productivity by preventing plant diseases, their interaction with biological systems reveals a complicated toxicity profile. For instance, the positive surface charges on iron oxide nanoparticles can lead to genotoxic and cytotoxic consequences through disruption of cell membrane permeability and non-specific interactions. Similarly, silver nanoparticles (Ag-NPs) have raised concerns due to their cytotoxic and genotoxic effects on various cell lines and their bioaccumulation in aquatic organisms, leading to physiological disruptions. However, some silver nanoparticles applications, such as biogenic Ag-NPs, have been shown to suppress harmful microorganisms beneficially, impacting soil bacterial communities and nitrogen cycling.

Other examples shows that the toxicity profile of copper-based nano-fungicides, particularly Cu₂O-NPs, has demonstrated high acute toxicity in zebrafish, suggesting their limited suitability as fungicides without thorough investigation into their environmental fate, bioavailability, and potential toxicity to non-target organisms (Yuan *et al.*, 2023). The observed toxicity may be attributed to the higher permeability of Cu²⁺ ions through the cytoplasmic membrane, although this alone does not fully explain the variance in antifungal activity observed among different Cu-based nanomaterials. This highlights the inherent challenge in distinguishing the toxicity of nanoparticles from that of the active substances they carry, as the environmental persistence of free ions, such as Cu²⁺, plays a significant role in toxicity, regardless of the nanoform.

Conversely, nano-formulations like chlorothalonil@mesoporous silica nanoparticles with β-glucans have been found to dramatically lower toxicity to aquatic species, illustrating how nano-agrochemicals can sometimes reduce toxicity compared to traditional formulations. Furthermore, organic polymers-based nanomaterials, such as lignin-derived carbon nanoparticles and pterostilbenes, have demonstrated minimal toxicity, supporting their safe and beneficial use against plant diseases.

The ecotoxicological impacts of nanocarriers are equally multifaceted. For example, the ecotoxicity of copper nanoparticles (Cu-NPs) was found to vary between bacterial strains, showing greater toxicity towards Gram-positive bacteria compared to Gram-negative, with a noted decrease in ecotoxicity correlating with increased hydrodynamic sizes of Cu-NPs for the Gram-negative strain. In contrast, the usage of copper-based soil improvers in nanoforms has been recognized for its reduced negative environmental effects compared to traditional copper-based agrochemicals, achieving targeted benefits with minimal ecological disruption.

Iron nanoparticles have been shown to be less toxic to aquatic life compared to other metal nanoparticles, advocating for their use in agriculture over alternatives like silver nanoparticles. However, high concentrations of iron-based nanoparticles have caused genotoxicity in maize seedlings, indicating potential toxicity hazards with prolonged exposure or larger doses.

Research into nano-insecticides and functionalized silica nanoparticles has highlighted the potential for precise pest control with minimal unintended consequences, demonstrating low environmental and physiological impacts. This careful balance is crucial for the development of safe and effective insecticidal techniques using nanomaterials.

Collectively, the toxicity and ecotoxicity of nanocarriers containing agrochemicals present a complex landscape of positive, negative, and neutral effects that are deeply influenced by the chemical type, concentration, and environmental context. These findings underscore the importance of thorough risk assessment and careful management in the application of nano-agrochemicals to harness their benefits while mitigating potential adverse effects.

5.2 Elaboration of recommendations to improve the quantity and quality of information

The following tables will report the elaboration of recommendations to improve the quantity and quality of information regarding conventional and nano-enabled plant protection products, biocidal products and fertilising products, both from a technical and from a legal perspective in regards to Biocidal Products Regulation (EU) 528/2012, Plant Protection Products Regulation (EC) No 1107/2009 and Fertilising products Regulation (EU) 2019/1009 to ensure the identification and safety of nanomaterial-containing substances and products. As the BPR is the only legislation among these that specifically addresses nanomaterials, the discussions on recommendations will begin with this regulation.

5.2.1 Biocidal Products Regulation (EU) 528/2012

TABLE 12 - BIOCIDAL PRODUCTS REGULATION (EU) [528/2012](#) (CONSOLIDATED VERSION: 15/04/2022)

Relevant provisions of the regulation	Evaluation and discussion drawn from the ELS
<p>Recital (66)</p> <p>There is scientific uncertainty about the safety of nanomaterials for human health, animal health and the environment. In order to ensure a high level of human health protection, free movement of goods and legal certainty for manufacturers, it is necessary to develop a uniform definition for nanomaterials, if possible based on the work of appropriate international forums and to specify that the approval of an active substance does not include the nanomaterial form, unless explicitly mentioned. The Commission should regularly review the provisions on nanomaterials in the light of scientific progress.</p>	<p>The provision outlined in Recital (66) remains highly pertinent in the current regulatory landscape, highlighting the ongoing scientific uncertainty surrounding the impact of nanomaterials on human health, animal health, and the environment. This underscores the need for a tailored definition of nanomaterials, for instance that consider nanoparticles smaller than 10nm more carefully, reflecting the critical need for clarity in ensuring consumer protection, facilitating the free movement of goods, and providing legal certainty for manufacturers. The explicit mention that the approval of an active substance does not automatically extend to its nanoform unless specifically stated, coupled with the directive for the Commission to conduct regular reviews of nanomaterial provisions, reinforces the regulation adaptability to evolving scientific insights.</p> <p>From the perspective of the ELS performed in this work, a potential update to this provision could include the integration of a precise definition for nano-agrochemicals, derived from the establishment of a specific database as recommended below. Despite the agricultural uses of biocides are out of the scope of the BPR Regulation (see here), this addition would not only refine the scope of the Regulation but it</p>

	<p>would also enhance its applicability and effectiveness in addressing the unique challenges posed by nano-agrochemicals. Furthermore, it is advisable to articulate the timeframe and frequency for the review of nanomaterial provisions explicitly. Instituting a clear schedule for these reviews would ensure that regulatory adjustments are timely, reflective of the latest scientific developments, and coherent with the dynamic nature of nanotechnology research and its applications.</p>
<p>Art. 3. 1. Definitions</p> <p>For the purposes of this Regulation, the following definitions shall apply:</p> <p>(z) ‘nanomaterial’ means a natural or manufactured active substance or non-active substance 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 1-100 nm.</p> <p>Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials.</p> <p>For the purposes of the definition of nanomaterial, ‘particle’, ‘agglomerate’ and ‘aggregate’ are defined as follows:</p> <ul style="list-style-type: none"> - ‘particle’ means a minute piece of matter with defined physical boundaries, - ‘agglomerate’ means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components, - ‘aggregate’ means a particle comprising strongly bound or fused particles; 	<p>The definition of 'nanomaterial' as specified in Article 3.1 of the Biocidal Products Regulation provides a foundational basis for identifying materials that fall within the scope of nanotechnology regulation. It encompasses natural or manufactured active and non-active substances that are in the form of particles, aggregates, or agglomerates, with a significant proportion of these particles having at least one external dimension in the size range of 1-100 nm. This definition also explicitly includes fullerenes, graphene flakes, and single-wall carbon nanotubes with dimensions below 1 nm as nanomaterials, offering a broad coverage of nanostructures.</p> <p>However, in light of advancements in the field of nanotechnology and its application in agriculture, as highlighted by the ELS, there is a compelling need to revise this definition to better capture the evolving landscape of nano-agrochemicals. This involves key updates:</p> <ul style="list-style-type: none"> - "Nano-agrochemical" definition: There is a notable absence of a specific definition of 'nano-agrochemicals' to indicate products that can contain any percentage of nanomaterial as defined by the EC Recommendation. Incorporating a definition that acknowledges nano-agrochemicals as chemical mixtures containing any percentage of a 'nanomaterial' in any form would significantly enhance the regulation's clarity and applicability to agricultural nanotechnologies. This inclusion would ensure that products are appropriately classified and regulated, reflecting their unique properties and potential impacts. - Complementary definitions. While it is acknowledged that the current EC

	<p>Recommendation already clearly define nanomaterials within the EU regulatory framework irrespective to their potential inherent hazardous properties or risks to human health and the environment, it is remarked that certain type of nanomaterials are indeed of particular concerns for toxicity and ecotoxicity point of views. For instance, quantum dots, typically smaller than 10 nm in size, constitute a crucial category of nanomaterials renowned for their ability to easily permeate biological membranes, thereby presenting potentially high toxic or ecotoxic effects that are difficult to predict. By explicitly incorporating dots into the definition of nanomaterials, the regulation would encompass a wider range of nanotechnologies, guaranteeing that these potent materials undergo thorough safety and efficacy evaluations.</p>
<p>Art. 3. 3. Definitions</p> <p>The Commission may, at the request of a Member State, decide, by means of implementing acts, whether a substance is a nanomaterial, having regard in particular to Commission Recommendation 2011/696/EU of 18 October 2011 on the definition of nanomaterial (list of substance), and whether a specific product or group of products is a biocidal product or a treated article or neither. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 82(3).</p>	<p>The provision under Article 3.3 remains a critical aspect of the regulatory framework, providing the EC with the authority to determine, through implementing acts, whether a substance qualifies as a nanomaterial. This determination takes into consideration the Commission Recommendation 2022/C 229/01, which offers a definition of the term nanomaterial, thereby ensuring a consistent and informed approach to regulation. Furthermore, the provision empowers the Commission to classify specific products or groups of products as biocidal products, treated articles, or neither, enhancing the regulation's flexibility and adaptability to evolving technological and market conditions.</p> <p>The ability to make these determinations at the request of a Member State ensures that the regulatory framework can respond dynamically to new scientific evidence and technological advancements. The process for adopting these implementing acts, as outlined in Article 82(3), guarantees that such decisions are made through a rigorous and transparent examination procedure, reinforcing the regulation's commitment to safeguarding public health and the environment while maintaining the free movement of goods within the EU.</p> <p>Given the provision's relevance and the importance of maintaining a robust mechanism</p>

	<p>for classifying nanomaterials and biocidal products, no changes to this provision are recommended.</p>
<p>Art. 3. 4. Definitions</p> <p>The Commission shall be empowered to adopt delegated acts in accordance with Article 83 in order to adapt the definition of nanomaterial set out in point (z) of paragraph 1 of this Article in view of technical and scientific progress and taking into account the Recommendation 2011/696/EU.</p>	<p>Article 3.4 provides the EC with the authority to adapt the definition of nanomaterials through the adoption of delegated acts, reflecting the necessity for regulatory frameworks to evolve in tandem with technical and scientific advancements. This adaptive mechanism, crucial for maintaining the regulation's relevance and effectiveness, is to be exercised in accordance with Article 83, ensuring that changes are rooted in a structured legislative process and consider the pivotal Commission Recommendation 2011/696/EU.</p> <p>The ELS supports the current applicability of this provision, underlining its significance in ensuring the regulatory definition of nanomaterials remains aligned with the latest scientific understanding and technological developments. However, it is proposed that the coordination in providing complementary definition of nano-agrochemicals should explicitly incorporate scientific opinions and evaluations from relevant authorities, particularly the ECHA and the EFSA. This collaborative approach would ensure that regulatory updates are informed by comprehensive risk assessments and the latest research findings, enhancing the safety and efficacy of nano-agrochemicals in the market. In addition, by integrating insights related to nano-agrochemicals, it is possible to expand the Commission's definition of nanomaterials, which currently does not consider potential inherent hazards or risks to human health and the environment.</p> <p>Furthermore, it is recommended that the Commission undertake to publish and regularly update a list of nano-enhanced chemicals, as outlined in the recommendations. Such a list/database would serve as a fundamental resource for stakeholders, from regulators to manufacturers and end-users, ensuring transparency and accessibility of information regarding nano-agrochemicals authorized for use within the EU.</p>
<p>Art. 4. 4. Conditions for approval</p>	<p>Article 4.4 succinctly addresses the conditions for the approval of active substances within the regulatory framework, clearly stipulating that such approval does not extend to nanomaterials</p>

The approval of an active substance shall not cover nanomaterials except where explicitly mentioned.

unless they are explicitly mentioned. This provision underscores the regulatory distinction between conventional active substances and their nanomaterial counterparts. A further distinction should be noted in terms of the BPR procedure, whereby an active ingredient can be approved if one safe application can be demonstrated. Subsequently, each product containing the active ingredient can be authorized based on an authorization dossier and evaluation. This two-level distinction highlights the need for specific considerations.

For the approval of the active ingredient, the Commission Recommendation 2022/C 229/01, offers already an actionable definition of the term nanomaterial, thereby ensuring a consistent and informed approach to regulation of active ingredient.

However, for the authorization of products, the insights drawn from the ELS suggest a pivotal update to this provision, reflecting the introduction of a nano-agrochemical definition, i.e. chemical products that are used in the agricultural field and embed nanomaterials with an external dimension in the nanoscale. This update necessitates a shift in the authorization process, advocating for an approach that considers nano-agrochemical products in their entirety rather than isolating the evaluation to specific nanomaterials. This recommendation arises from the evidence indicating that the efficacy, toxicity, and ecotoxicity of nano-agrochemicals are intrinsically linked to the synergistic interplay between the carriers, the active substances they deliver and other components of a formulated product. Thus, given the complexity and diversity of nano-agrochemical product formulations, a generalized approach without the definition of nano-agrochemical proves insufficient and potentially misleading in terms of confounding approval and authorization processes. The proposed revision aims to ensure that regulatory authorization of nano-agrochemical is informed by a comprehensive assessment of these integrated systems. This holistic evaluation is crucial for accurately determining the safety, efficacy, and environmental impact of these products.

Art. 19. 1 Conditions for granting an authorisation

A biocidal product other than those eligible for the simplified authorisation procedure in accordance with Article 25 shall be authorised provided the following conditions are met:

(f) where nanomaterials are used in that product, the risk to human health, animal health and the environment has been assessed separately.

Article 19.1(f) of the Biocidal Products Regulation mandates a separate assessment of the risks posed to human health, animal health, and the environment by nanomaterials used in biocidal products, a provision that underscores the unique considerations associated with nanotechnology. This requirement is fundamental in ensuring that the potential impacts of nanomaterials are thoroughly evaluated, reflecting the regulation's commitment to safeguarding human and environmental health.

The insights collected from the ELS suggest that the processes of approval of nanomaterial active ingredients as well as the authorization of nano-agrochemicals containing nanomaterials, advocate for information inclusion in a specific EU-level database. Such a database would serve as a centralized repository of information, enhancing transparency and facilitating regulatory oversight (see recommendations below). Furthermore, the development of standardized instructions for use, tailored to nano-agrochemical products, is recommended to provide clear guidance on the safe and effective application of these products, addressing both general and specific use scenarios.

Additionally, the health and environmental risk assessment process for nano-agrochemicals should explicitly incorporate specific toxicity and ecotoxicity testing, as highlighted in the recommendations below. This approach ensures a more comprehensive understanding of the potential impacts of nano-agrochemicals, enabling a more informed assessment of their safety.

Crucially, it is proposed to include in this section with the requirement for substantiating efficacy claims of nano-agrochemicals in comparison to their non-nano counterparts. This could be achieved through detailed literature reviews or specific comparative testing, offering evidence of the added value or enhanced performance provided by nano-enabled formulations. Such comparative analysis is vital in justifying the use of nano-agrochemicals, ensuring that their adoption is based on clear, demonstrable benefits over traditional formulations.

<p>Art. 25. Eligibility for the simplified authorisation procedure</p> <p>For eligible biocidal products, an application for authorisation may be made under a simplified authorisation procedure. A biocidal product shall be eligible if all the following conditions are met:</p> <p>(c) the biocidal product does not contain any nanomaterials;</p>	<p>Article 25 outlines the criteria for biocidal products to be eligible for a simplified authorisation procedure, specifically noting that a product must not contain any nanomaterials to qualify. This criterion underscores the regulatory approach to nanomaterials as requiring more comprehensive assessment due to their unique properties and potential impacts.</p> <p>Given the advancements in the field of nanotechnology and the emergence of nano-agrochemicals, it is pertinent to update this provision to reflect the nuanced understanding and classification of these products. A revision is suggested to specify that "the biocidal product is not a nano-agrochemical," in alignment with the proposal to include a definition of nano-agrochemicals.</p>
<p>Art. 58.3. Placing on the market of treated articles</p> <p>The person responsible for the placing on the market of such a treated article shall ensure that the label provides the information listed in the second subparagraph, where:</p> <p>(d) the name of all nanomaterials contained in the biocidal products, followed by the word 'nano' in brackets;</p>	<p>Article 58.3 addresses the requirements for labelling treated articles placed on the market, specifically mandating the disclosure of any nanomaterials used in the biocidal products treating those articles, with an explicit mention of the term 'nano' in brackets following the name of the nanomaterials. This provision plays a crucial role in ensuring transparency and informing consumers about the presence of nanomaterials in treated articles, which is increasingly important given the growing use of nanotechnology in product formulations.</p> <p>Based on the insights from the ELS, it is proposed that this provision be revised to enhance its specificity and utility in the context of nano-agrochemicals. The suggested update would require the disclosure of the complete defined composition of the nano-agrochemical, as outlined in a specific notification system for manufacturers or importers. This system, as proposed below, aims to streamline the regulatory process by providing a centralized platform for the submission and verification of comprehensive data on nano-agrochemicals.</p> <p>Furthermore, the labelling requirements should be expanded to include a clear reference to the nano-agrochemical including detailed instructions for use, ensuring that end-users are fully informed about the safe and effective application of the treated articles. This reference should also link the label information to a specific database of nano-agrochemicals at the EU level</p>

	<p>that would facilitate access to in-depth information about nanomaterials used, including their safety profiles, and regulatory status. This database would serve as a valuable resource for both regulators and consumers, promoting greater understanding and confidence in the use of nano-enabled products.</p>
<p>Art. 65.3. Compliance with requirements</p> <p>Every five years, from 1 September 2015, Member States shall submit to the Commission a report on the implementation of this Regulation in their respective territories. The report shall include in particular:</p> <p>(d) information on the use of nanomaterials in biocidal products and the potential risks thereof.;</p>	<p>Article 65.3 mandates that MS report to the Commission every five years on the implementation of the Biocidal Products Regulation within their territories, with a specific requirement to include information on the use of nanomaterials in biocidal products and the assessment of potential risks associated with their use. This provision is critical for monitoring the integration of nanotechnology in biocidal products and ensuring ongoing compliance with regulatory standards aimed at safeguarding public and environmental health.</p> <p>The ELS reveals a significant volume of documentation on nanomaterials and a marked increase in interest within the market for nano-agrochemicals. Given the rapid pace of technological advancement and the evolving landscape of nanomaterials application in biocidal products, the current five-year reporting interval may not sufficiently capture the dynamic nature of nanomaterials use and associated risk profiles.</p> <p>Therefore, it is recommended that the reporting frequency be revised to a more frequent interval, specifically every three years. This adjustment would allow for more timely updates on the use of nanomaterials, enabling both the Commission and MS to respond more effectively to new scientific evidence and market trends. It would also enhance the regulatory framework's ability to adapt to technological advancements, ensuring that risk assessments and regulatory measures remain aligned with the latest developments in nanotechnology.</p> <p>Such an amendment would underscore the EU's commitment to proactive and responsive regulation of nanomaterials in biocidal products, reinforcing the overarching objectives of consumer protection, environmental preservation, and innovation support within the biocidal products sector.</p>

Art. 69.2. Classification, packaging and labelling of biocidal products

In addition to compliance with paragraph 1, authorisation holders shall ensure that labels are not misleading in respect of the risks from the product to human health, animal health or the environment or its efficacy and, in any case, do not mention the indications 'low-risk biocidal product', 'non-toxic', 'harmless', 'natural', 'environmentally friendly', 'animal friendly' or similar indications. In addition, the label must show clearly and indelibly the following information:

(b) the nanomaterials contained in the product, if any, and any specific related risks, and, following each reference to nanomaterials, the word 'nano' in brackets;

Article 69.2 emphasizes the importance of accurate and non-misleading labelling for biocidal products, particularly in conveying the risks associated with their use to human health, animal health, or the environment, as well as their efficacy. It strictly prohibits the use of terms that may understate the risks, such as 'low-risk biocidal product', 'non-toxic', 'harmless', 'natural', 'environmentally friendly', 'animal friendly', or similar indications. Moreover, it mandates the clear and indelible disclosure of any nanomaterials present in the product, alongside any specific risks associated with these materials, with the term 'nano' explicitly mentioned in brackets following each reference to nanomaterials.

Given the insights obtained from ELS and the increasing market interest in nano-agrochemicals, this provision's relevance is reaffirmed. However, to enhance the provision's effectiveness in the context of modern technological advancements and to improve consumer access to detailed product information, it is suggested that labelling requirements be expanded to include traceability references. These could take the form of QR codes or barcodes that, when scanned, direct users to the comprehensive database of nano-agrochemicals at the EU level, as proposed below. This database would provide in-depth information on the composition, safety, and regulatory status of nano-agrochemicals contained within the product.

Incorporating such traceability references into the labelling would significantly augment the provision's aim of ensuring transparency and safety.

Annex II. Information requirements for active substances.

5. Tests submitted for the purpose of the approval of an active substance shall be conducted according to the methods described in Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (1). However, if a method is inappropriate or not described, other methods

Annex II of the regulation sets forth the information requirements for the approval of active substances, stipulating that tests must adhere to the methodologies outlined in Commission Regulation (EC) No 440/2008, in alignment with REACH. This ensures a standardized approach to the evaluation of chemical substances, including nanomaterials. However, it acknowledges the potential need for alternative methods that are scientifically appropriate and, where possible, internationally recognized, particularly when established methods are unsuitable or inadequately

shall be used which are scientifically appropriate, whenever possible internationally recognised, and their appropriateness must be justified in the application. When test methods are applied to nanomaterials, an explanation shall be provided of their scientific appropriateness for nanomaterials, and where applicable, of the technical adaptations/adjustments that have been made in order to respond to the specific characteristics of these materials.

described for specific substances. The provision further requires that when tests are applied to nanomaterials, there must be a clear justification of the scientific appropriateness of the chosen methods for these materials, including any necessary technical modifications to accommodate the unique properties of nanomaterials.

Given the unique challenges and opportunities presented by nano-agrochemicals, as highlighted by the ELS, it is proposed that this provision be enhanced to include specific advices for testing nano-agrochemicals. This improvement should mandate that explanations for the scientific appropriateness of test methods for nano-agrochemicals be sustained by robust literature support. This support should detail the efficacy of specific methods for evaluating the health and environmental impacts of nano-agrochemicals, underlining the importance of evidence-based method selection.

Moreover, the provision should require that comparative studies between nano-agrochemical forms and their non-nano counterparts be conducted and submitted as part of the approval process. These studies are essential for demonstrating the added value or enhanced efficacy of nano-agrochemicals, providing a clear basis for their approval and use. Such comparative analyses would contribute significantly to understanding the benefits and potential risks of nano-agrochemicals, enabling regulators and stakeholders to make informed decisions regarding their deployment in agricultural and other settings.

Incorporating these recommendations into the regulation would strengthen the framework for assessing nano-agrochemicals, ensuring an evaluation process both rigorous and reflective of the latest scientific advancements.

Annex III. Information requirements for biocidal products.

5. Tests submitted for the purpose of authorisation shall be conducted according to the methods described in Regulation (EC) No 440/2008. However, if a method is inappropriate or not described, other methods shall be used which are scientifically appropriate, whenever

Annex III of the regulation delineates the information requirements for the authorization of biocidal products, mirroring the stipulations set forth in Annex II regarding the necessity for tests to conform to the methodologies described in Regulation (EC) No 440/2008. This ensures that the assessment of biocidal products, including those containing nanomaterials, is underpinned by standardized and scientifically

possible internationally recognised, and their appropriateness must be justified in the application. When test methods are applied to nanomaterials, an explanation shall be provided of their scientific appropriateness for nanomaterials, and, where applicable, of the technical adaptations/adjustments that have been made in order to respond to the specific characteristics of these materials.

robust testing methods. The provision acknowledges the potential need for alternative testing methods when standard approaches are deemed inappropriate for specific substances, emphasizing the requirement for these alternatives to be scientifically appropriate and internationally recognized, with their selection justified within the application process. The inclusion of a clause specific to nanomaterials reflects an understanding of the unique challenges posed by these substances. It mandates that any test methods applied to nanomaterials must be accompanied by a thorough explanation of their scientific suitability for these materials, including any necessary modifications to address their distinctive properties. This requirement is crucial for ensuring that the evaluation of nanomaterials within biocidal products is both accurate and reflective of their potential impacts on human health and the environment.

Given the insights from the ELS, the evaluation and discussion applicable to this provision would closely resemble that provided for Annex II. It is recommended that the regulation be further refined to explicitly require comprehensive literature support for the selected testing methods when evaluating nano-agrochemicals. This should include evidence of the methods' effectiveness in assessing health and environmental effects specific to nanomaterials. Additionally, the provision should mandate the submission of comparative studies between nano-agrochemicals and their non-nano counterparts, aiming to elucidate the comparative advantages or enhanced efficacy of nano-agrochemicals. These comparative analyses are vital for substantiating the authorization of nano-agrochemicals, ensuring that their use is justified based on a demonstrable benefit over traditional formulations.

Annex VI. Common principles for the evaluation of dossiers for biocidal products. Introduction.

2. The principles set out in this Annex can be applied in their entirety to the evaluation of biocidal products comprised of chemical substances. For biocidal products containing micro-organisms, these principles should be further developed in technical guidance taking

Annex VI outlines the foundational principles for evaluating dossiers of biocidal products, emphasizing that these principles are fully applicable to products comprised of chemical substances. It acknowledges the need for additional development and adaptation of these principles for biocidal products containing micro-organisms or nanomaterials, suggesting that the unique characteristics and implications of these materials require specialized

into account practical experience gained, and be applied taking into account the nature of the product and the latest scientific information. In the case of biocidal products containing nanomaterials, the principles set out in this Annex will also need to be adapted and elaborated in technical guidance to take account of the latest scientific information.

consideration. Specifically, for biocidal products containing nanomaterials, the annex underscores the necessity to adapt and elaborate on the established principles through technical guidance, which should incorporate the latest scientific information available.

A similar commentary applies as with the previous points discussed. The rapidly evolving field of nanotechnology and the increasing integration of nanomaterials into biocidal products necessitate a dynamic and informed approach to regulatory evaluation. It is recommended that the principles for evaluating biocidal products containing nanomaterials be continuously updated and refined through the development of specific technical guidance. This guidance should be grounded in the most current scientific research and practical experience, ensuring that the regulatory framework remains at the forefront of technological advancements. Furthermore, this technical guidance should explicitly address the complexities associated with assessing the safety, efficacy, and environmental impact of nano-enhanced biocidal substances. It should include detailed methodologies for toxicity and ecotoxicity testing, as well as comparative assessments to non-nano forms, providing a comprehensive framework for understanding the unique attributes and potential risks of nanomaterials. By incorporating such detailed and up-to-date scientific insights, regulatory authorities can better ensure the safe and effective use of nano-enabled biocidal products, fostering innovation while protecting public health and the environment.

5.2.2 Plant Protection Products Regulation (EC) No 1107/2009

Surprisingly, the Plant Protection Products Regulation (EC) No 1107/2009 does not contain any specific provisions in reference to nanoforms, neither as carriers nor when loaded with agrochemicals. This omission is notable, especially considering the increasing prevalence and significance of nanotechnology in the agricultural sector. The regulation, which plays a crucial role in ensuring the safety and efficacy of plant protection products, lacks provisions for the evaluation, authorization, and labelling of nano-enabled agrochemicals that are notably embedded in guidelines from EFSA (EFSA Scientific Committee *et al.*, 2021). This gap underscores a pressing need for the regulation to evolve in response to technological advancements, ensuring comprehensive oversight of nano-agrochemicals.

The insights drawn from the Biocidal Products Regulation (EU) 528/2012 provide a valuable framework that could inform the necessary updates to the Plant Protection Products Regulation. The Biocidal Products Regulation addresses several key aspects of nanomaterial regulation, including the

need for a uniform definition of nanomaterials, the requirement for specific labelling of products containing nanomaterials, and the obligation to assess the risks posed by nanomaterials to human health, animal health, and the environment separately.

For instance, the Biocidal Products Regulation mandates that the approval of an active substance does not automatically extend to its nanomaterial form unless explicitly mentioned, highlighting the unique considerations required for nanoforms. Similarly, it requires labels to clearly indicate the presence of nanomaterials, ensuring transparency and informed decision-making by consumers. Furthermore, the regulation emphasizes the importance of adapting evaluation principles and testing methods to account for the specific characteristics of nanomaterials, ensuring that their safety and efficacy are thoroughly assessed.

These considerations are equally applicable to the Plant Protection Products Regulation. For example, the introduction of a specific definition for "nano-agrochemicals" could enhance clarity and regulatory precision, acknowledging the distinct properties and potential impacts of these products. The adaptation of testing methods to evaluate the health and environmental effects of nano-agrochemicals, supported by robust literature and comparative studies to non-nanoforms, would provide a comprehensive understanding of their safety and enhanced efficacy. Additionally, the establishment of a specific EU-level database for nano-agrochemicals and the inclusion of traceability references (e.g., QR codes or barcodes) on product labels could improve transparency and accessibility of information.

The omission of provisions related to nanoforms in the Plant Protection Products Regulation suggests a significant opportunity for regulatory evolution. By integrating lessons learned from the Biocidal Products Regulation and addressing the unique challenges posed by nano-agrochemicals, the regulation can better safeguard public health and the environment while supporting innovation in agricultural practices. The implementation of these recommendations would ensure that plant protection products containing nanomaterials are subject to a regulatory framework that is both rigorous and responsive to the latest scientific and technological developments.

5.2.3 *Fertilising products Regulation (EU) 2019/1009*

Similar to the Plant Protection Products Regulation (EC) No 1107/2009, the Fertilising Products Regulation (EU) 2019/1009 also notably lacks specific provisions addressing nanoforms, both as carriers and when loaded with agrochemicals. This absence is particularly striking given the potential for nanotechnology to significantly enhance the effectiveness and environmental compatibility of fertilising products.

Drawing from the discussions around the Biocidal Products Regulation (EU) 528/2012, it becomes evident that several key regulatory aspects could beneficially be applied to the Fertilising Products Regulation to ensure comprehensive oversight of nano-enabled fertilising products. These include the necessity for a clear definition of nano-agrochemicals, specific labelling requirements to indicate the presence of nanomaterials, and detailed risk assessments tailored to the unique properties of nanomaterials.

The introduction of amendments to explicitly address nanoforms within the Fertilising Products Regulation could significantly enhance the regulatory framework. By adopting a definition for "nano-agrochemicals" that reflects their distinct characteristics, ensuring labels provide transparent information about nanomaterial content, and mandating thorough safety and efficacy evaluations, the regulation can better protect public health and the environment. Additionally, creating a dedicated EU-level database for nano-agrochemicals and incorporating traceability references on product labels would further improve transparency and facilitate access to comprehensive product information.

The omission of nanoform-specific provisions in the Fertilising Products Regulation highlights a critical area for regulatory development. Adapting the regulation to include explicit guidelines for nano-enabled fertilising products would align it more closely with current scientific and technological advancements, fostering safer and more innovative agricultural practices.

5.3 Recommendations on alternative approaches

5.3.1 Inclusion of specific provisions in the current regulations

It is observed that Regulations (EC) No 1107/2009 and (EU) 2019/1009 currently lack explicit considerations pertaining to nano-agrochemicals, a gap that stands in contrast to Regulation (EU) No 528/2012, which incorporates specific provisions regarding nano-enhanced chemicals. Given the escalating influence of nano-agrochemicals, as substantiated by the ELS, it is imperative that all relevant regulations are amended to include comprehensive considerations for nano-enhanced chemicals within these domains. This incorporation could be effectively driven by guidelines published by EFSA in 2021 (EFSA Scientific Committee *et al.*, 2021).

5.3.2 Create a framework for standardized instruction of use

There exists a pressing need to establish a uniform framework for the provision of standardized instructions for the use of nano-agrochemicals. Manufacturers, who are adept in handling scientific data concerning chemicals and carriers, develop these products. Nonetheless, downstream users, encompassing farmers and professionals within the agro-sciences sector, may not be privy to the same depth of information.

It is thus recommended that a comprehensive framework be instituted, mandating manufacturers to compile detailed instructions for use in downstream scenarios. Such instructions should encompass a variety of use-case scenarios in the field, taking into account the diverse applications of nanocarriers containing identical active substances intended for multiple uses. For instance, if a nanocarrier with the same active ingredient is designed for two distinct purposes, the instructions should delineate separate scenarios for each application. Furthermore, these instructions for use must undergo approval by competent national or European Union authorities prior to their market introduction. This measure will ensure that all stakeholders have access to consistent, clear, and authoritative guidance on the safe and effective application of nano-agrochemicals, thereby mitigating risks associated with toxicity and ecotoxicity, and enhancing the regulatory oversight of these innovative products.

5.3.3 Create a specific database of nano-agrochemicals at EU level

In alignment with advancing regulatory frameworks and enhancing transparency within the domain of nano-agrochemicals, the establishment of a specialised database at the EU level is proposed. This database, to be curated by EUON under the auspices of the ECHA, should ideally be developed in collaboration with interagency efforts, including EFSA. Notably, MS initiatives like France's decree no. 2012-232 (see <https://www.r-nano.fr/>), which mandates an annual declaration on substances at nanoscale, could serve as a model for the proposed database. Indeed, the integration of such national initiatives into the EU-wide database would enhance the collective understanding of nanomaterials, their uses, and their market dynamics.

The primary objective of this initiative is to compile and centralise essential information pertaining to nano-agrochemicals, thereby facilitating access for regulators, researchers, and stakeholders. The proposed database should meticulously catalogue nano-agrochemicals, taking into consideration various critical aspects such as their intended use, the chemical nature of both the active substances or formulations containing nanoforms and nanocarriers. Moreover, the database should feature links to specific regulations governing the use of these nano-agrochemicals, thereby ensuring regulatory compliance and facilitating the seamless integration of this information with existing data repositories. This includes the possibility of interlinking with the chemical information provided in IUCLID or other

relevant databases. Such a comprehensive repository would not only streamline the regulatory process but also enhance the safety and efficacy of nano-agrochemicals application across the EU. By providing a central point of reference for detailed information on the composition, usage, and regulatory status of nano-agrochemicals, the database would significantly contribute to informed decision-making and foster a safer, more sustainable agricultural sector.

5.3.4 *Notification system for manufacturers or importers*

To enhance regulatory oversight and ensure compliance within the nano-agrochemicals sector, the establishment of a comprehensive notification system for manufacturers and importers is recommended. This system would mandate the disclosure of formulations linked to the identification numbers within the proposed EU-wide database of nano-agrochemicals. Drawing inspiration from successful regulatory frameworks, such as the Tobacco Products Directive (2014/40/EU), the creation of an EU Common Entry Gate (EU-CEG) for nano-agrochemicals is proposed.

The EU-CEG for nano-agrochemicals would require manufacturers and importers to submit essential information to the regulatory authorities in the Member States where they intend to market their products. This submission process necessitates the development of an IT tool by the EC, designed to streamline the provision of this data. Such a tool would not only facilitate the notification process but also serve as a platform for the submission of standardized instructions for use, aligning with the recommendations outlined in the previous section. This proactive approach aims to reinforce the traceability of nano-agrochemicals across the EU, ensuring that all products entering the market are duly registered and their safety and compliance information is readily accessible. By leveraging technology to create a unified submission portal, the EC can significantly improve the efficiency of regulatory processes, enhance the safety of agricultural practices, and provide clarity and certainty for manufacturers, importers, and regulators alike.

5.3.5 *Specific literature searches to support efficacy claims*

The undertaking of systematic literature reviews is deemed essential to provide regulators with accurate and up-to-date information necessary for evaluating the comparative efficacy of nano-enabled versus conventional agrochemicals. Despite the anticipation that existing data may often be insufficient for a comprehensive comparative assessment, these reviews are crucial in highlighting the potential advancements nano-enabled products may offer in terms of agrochemical efficacy.

Acknowledging the existence of information gaps, particularly concerning toxicity and ecotoxicity parameters, it is pivotal that such literature searches are also leveraged to substantiate claims regarding the efficacy of nano-agrochemicals in comparison to their non-nano counterparts. In fact, it is remarked that market authorization for new nano-agrochemical products be predicated upon the demonstration of a distinct and unequivocal advantage over existing solutions. This criterion ensures that the introduction of nano-agrochemicals into the market is justified not only by their innovative nature but also by their ability to offer clear benefits, thereby reinforcing the principles of safety, sustainability, and enhanced agricultural productivity.

In light of these considerations, a structured framework for conducting and evaluating literature reviews should be established and embedded in the regulations discussed. This framework would guide the assessment process, ensuring that efficacy claims are supported by robust scientific evidence, thereby facilitating informed regulatory decisions and promoting the responsible development and use of nano-agrochemicals.

5.3.6 *Mandating the submission of toxicity and ecotoxicity tests*

The toxicity and ecotoxicity of nano-agrochemicals may manifest through multiple mechanisms, with their physicochemical characteristics—such as size, morphology, surface charge, and chemical composition—playing a crucial role in determining their interaction with biological systems. The

generation of reactive oxygen species (ROS) is a key mechanism contributing to the (eco)toxicological profile of nano-agrochemicals, leading to oxidative stress, inflammation, and potential cellular damage including necrosis, accelerated apoptosis, or carcinogenesis. Evaluating these properties is indispensable for understanding the comprehensive (eco)toxicity profile of nano-agrochemicals, yet the complexity of biological systems often obscures direct causality.

To this end, the establishment of comprehensive testing criteria for assessing both the toxicity and ecotoxicity of nano-agrochemicals is essential to address the multifaceted challenge posed by their varied physicochemical properties and their potential impacts on efficacy, toxicity, and ecotoxicity profiles. Criteria for the selection of suitable testing methodologies must be rooted in robust validation, demonstrated through a minimum number of scientific publications that illustrate the method's adaptability to nanomaterials in the context of agrochemicals. These methodologies should, for instance, employ organisms conducive to Simple and Rapid Ecotoxicity and Toxicity Testing (SRETT), with a test duration possibly not exceeding 30 days, without the need for specialized equipment, cost-effective, and amenable to high-throughput screening optimization.

Authoritative bodies such as the OECD, ECHA, EPA and ISO have outlined standard assays that meet these criteria, encompassing a range of tests on algae, duckweed, amphipods, daphnids, chironomids, terrestrial plants, nematodes, earthworms, and accessible models such as zebrafish. The diversity of these tests underlines the complex interactions that nano-agrochemical can have with various organisms, resulting in a spectrum of effects including behavioural, morphological, cellular, molecular, and genetic alterations.

From the operative point of view, given the nanoparticles' potential for public health risks due to their catalytic activity and propensity for physiological process disruption upon entry into the body via various routes, it is imperative to employ a combination of assays. For instance, these should range from endotoxin and lactate dehydrogenase signalling to apoptosis and oxidative stress detection, to adequately identify biomarkers of nanoparticle-induced cellular damage. The challenge extends to the adaptation of traditional in vitro assays to accurately reflect the unique interactions of nanoparticles within biological environments, necessitating the development of novel methodologies.

Therefore, the requirement for the submission of consolidated test results that encompass a broad spectrum of organisms and biological impacts is critical. This holistic approach to the evaluation of nano-agrochemicals' toxicity and ecotoxicity profiles will ensure rigorous environmental and health safety assessments, facilitating informed regulatory decisions and safeguarding ecological integrity and public health. In advancing these testing criteria, a pivotal step is taken towards a nuanced understanding and regulation of nano-agrochemicals, aligning with the goal of maximizing agricultural productivity while minimizing potential adverse effects on health and the environment.

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7 Abbreviations

Acronym	Definition
2,4-D	2,4-dichlorophenoxyacetic Acid
2D	2 dimensional
3D	3 dimensional
A(1-5)	Area of Interest
A.EO	<i>Achillea millefolium</i> L. Essential Oil
AF4	Asymmetric Flow Field Flow Fractionation
AFM	Atomic Force Microscopy
Ag	Silver
Ag ₂ O	Silver Oxide
AgB	Silver-boron

Al	Aluminium
AMF	Arbuscular Mycorrhizal Fungi
Art.	Article
Au	Gold
BET	Brunauer-Emmett-Teller
BNP	Biochar Nanoparticle
BPR	Biocidal Products Regulation (EU) 528/2012
CaC	Calcium Carbonate
CAP	Captan
CAS	Chemical Abstract Service
CD	Carbon Dot
CeO ₂	Cerium Oxide
CLS	Classical Least Squares
CNR	Italian National Research Council
CNT-OH	Carbon Nanotubes Hydroxyl
CO	Clove Oil
CO ₂	Carbon Dioxide
Co ₃ O ₄	Cobalt Oxide
CoFe ₂ O ₄	Cobalt Ferrite
CQD	Carbon Quantum Dot
CS	Chitosan
CSNP	Chitosan Nanoparticle
CSO	Civil Society Organization
CSV	Comma Separated Value
CTAC	Cetyltrimethylammonium Chloride
Cu	Copper
Cu(OH) ₂	Copper Hydroxide
Cu ₂ (OH) ₂ CO ₃	Basic Copper Carbonate
Cu ₃ (PO ₄) ₂	Copper Phosphate
CuO	Copper Oxide
CuS	Copper Sulphide
CuSiNG	Copper Loaded Silica Nanogel
CuSO ₄	Copper Sulphate
DHA	Dehydrogenases
DiS-NH ₂	Disulfide Aminophenoxazinone
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
DOI	Digital Object Identifier
dsRNA	Double-stranded Ribonucleic Acid
EC	European Commission
EC	Exclusion Criteria
EC(1-5)	Exclusion Criteria Code Number
ECHA	European Chemicals Agency
EDTA	Ethylenediaminetetraacetic Acid
EFFA	European Flavour Association
EFSA	European Food Safety Authority
ELS	Extensive Literature Search
EPA	United States Environmental Protection Agency
EU	European Union
EU-CEG	European Union Common Entry Gate

EUON	European Union Observatory for Nanomaterials
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
FDA	United States Food and Drug Administration
Fe	Iron
Fe ₂ O ₃	Hematite
Fe ₃ O ₄	Magnetite
FeS	Iron Sulphide
FFF	Field Flow Fractionation
FPR	Fertilising Products Regulation (EU) 2019/1009
FSA	Food Standards Agency
FSANZ	Food Standards Australia New Zealand
GLY	Glyphosate
GO	Graphene Oxide
GOD	Glucose Oxidase
H ₂ O	Water
HA	Hydroxyapatite
ha	Hectare
Hal	Halloysite
IFOAM	International Federation of Organic Agriculture Movements
IR	Infrared
ISO	International Organization for Standardization
ISOF	Institute for the Organic Synthesis and Photoreactivity
IT	Information Technology
IUCLID	International Uniform Chemical Information Database
K	Potassium
LC ₅₀	Lethal Dose 50%
LC ₉₀	Lethal Dose 90%
L-CYN	Lambda-cyhalothrin
LDH	Layered Double Hydroxide
LGO	Lemongrass Oil
LNE	French National Metrology Lab
MAL	Multiangle Light Scattering
MBC	Microbial Biomass
METO	Metolachlor
MgO	Magnesium Oxide
Mn	Manganese
Mn ₂ O ₃	Manganese Dioxide
MnO	Manganese Oxide
MON	Mesoporous Organosilica
MoS ₂	Molybdenum Sulphide
MSN	Mesoporous Silica
MV	Mixed Valence
MWCNT	Multi-Walled Carbon Nanotube
N	Nitrogen
N ₂ O	Nitrous oxide
Na	Sodium
nano-U-NPK	Calcium Phosphate Nanoparticle doped with Potassium, Nitrate and Urea
NC	Nanocomposite
NCNT	Nitrogen-doped Carbon Nanotube

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NFM	Nanofibrous Membrane
NGO	Non-governmental Organization
NH ₃	Ammonia
Ni	Nickel
NIR	Near-Infrared
nm	Nanometer
NOAEL	No Observed Adverse Effect Levels
NP	Nanoparticle
NPK	Nitrogen, Phosphorous and Potassium
NTA	Non-targeted analysis
NUNE	Neem Urea Nano-emulsion
NZCF	Nano Zeolite Composite Fertilisers
NZNM	Nano Zeolite Based Nanomaterial
OECD	Organisation for Economic Co-operation and Development
PDF	Portable Document Format
PECO	Populations, Exposure, Comparators, Outcomes
PEG	Polyethylene Glycol
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
Ph.D.	Doctor of Philosophy
PHS	Porous Hollow Silica
PICO	Populations, Interventions, Comparators, Outcomes
PMAA	Poly(methacrylic Acid)
PPPR	Plant Protection Products Regulation (EC) No 1107/2009
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRO	Prochloraz
Pro	Proline
PROSPERO	International Prospective Register of Systematic Reviews
PSII	Photosystem II
PSWNT	Polyetherimide-modified Functionalized Carboxylated Single Walled Carbon Nanotube
QD	Quantum Dot
QR code	Quick-response Code
R&D	Research and Development
RCOM	Response to Comments Document
REACH	Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals
RH	Rice Husk
RIS	Research Information Systems
RNA	Ribonucleic Acid
RNAi	Ribonucleic Acid Interference
ROS	Reactive Oxygen Species
S	Sulphur
SA	Salicylic Acid
Se	Selenium
SEM	Scanning Electron Microscopy
SFA	Singapore Food Agency
Si	Silicon
SiO ₂	Silica
SNNM	Surface Nanostructured Nanomaterial
sp-ICP-MS	Single Particle Inductively Coupled Plasma Mass Spectrometry

SPc	Star Polycation Complex
SRETT	Simple and Rapid Ecotoxicity and Toxicity Testing
SRF	Slow-release Fertiliser
TBCC	Tribasic Copper Chloride
TCNP	Thiamine-chitosan Nanoparticle
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
TiO ₂	Titanium Dioxide
TMGMV	Tobacco Mild Green Mosaic Virus
TNU	Urea nanoparticle
TOF-SIM	Time-of-Flight Secondary Ion Mass Spectrometry
URL	Uniform Resource Identifier
UV	Ultraviolet
WHO	World Health Organization
WoS_CC	Web of Science_Core Collection
XRD	X-ray Diffraction
ZIF	Zeolitic Imidazolate Framework
Zn	Zinc
ZNCPC	Zincate Nano-clay Polymer Composite
ZnO	Zinc Oxide
ZnS	Zinc Sulphide
ZnSO ₄	Zinc Sulphate
ZrO	Zirconium Oxide
γ-CD	γ-cyclodextrin

8 Annexes

8.1 Annex I

Queries Plant Protection Product SciFinder – CAS number research only APPROVED from online database

[Registro de Productos Fitosanitarios \(mapa.gob.es\)](http://mapa.gob.es)

(112-30-1 OR 36729-58-5 OR 70084-71-8 OR 85566-12-7 OR 66455-17-2 OR 3100-04-7 OR 86-86-2 OR 86-87-3 OR 571-58-4 OR 67233-85-6 OR 1214-39-7 OR 148-24-3 OR 65195-55-3 OR 8042-47-5 OR 64742-46-7 OR 72623-86-0 OR 97862-82-3 OR 57960-19-7 OR 135410-20-7 OR 160430-64-8 OR 64-19-7 OR 68475-71-8 OR 77671-22-8 OR 463-83-2 OR 50-81-7 OR 6730-29-6 OR 53262-66-1 OR 62624-30-0 OR 21293-29-8 OR 65-85-0 OR 2905-69-3 OR 68214-43-7 OR 77-06-5 OR 112-05-0 OR 67701-09-1) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

(124-07-2 OR 334-48-5 OR 143-07-7 OR 112-80-1 OR 85566-26-3 OR 111-11-5 OR 110-42-9 OR 1511720-78-7 OR 74070-46-5 OR 211504-93-7 OR 72204-44-5 OR 67375-30-8 OR 865318-97-4 OR 120923-37-7 OR 150114-71-9 OR 348635-87-0 OR 11141-17-6 OR 131860-33-8 OR 231-722-6 OR 113614-08-7 OR 98243-83-5 OR 99283-01-9 OR 25057-89-0 OR 413615-35-7 OR 1072957-71-1 OR 149877-41-8) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

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(42576-02-3 OR 581809-46-3 OR 188425-85-6 OR 116255-48-2 OR 41483-43-6 OR 69327-76-0 OR 133-06-2 OR 471-34-1 OR 298-14-6 OR 75-20-7 OR 128639-02-1 OR 6485-40-1 OR 736994-63-1 OR 120116-88-3 OR 101205-02-1 OR 180409-60-3 OR 400882-07-7 OR 122008-85-9 OR 57966-95-7 OR 52315-07-8 OR 121552-61-2 OR 99129-21-2 OR 105512-06-9 OR 74115-24-5 OR 81777-89-1 OR 1702-17-6 OR 500008-45-7 OR 593-81-7 OR 7003-89-6 OR 999-81-5 OR 15545-48-9 OR 1596-84-5 OR 533-74-4) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

(52918-63-5 OR 1918-00-9 OR 40843-25-2 OR 120-36-5 OR 119446-68-3 OR 83164-33-4 OR 50563-36-5 OR 87674-68-8 OR 110488-70-5 OR 149961-52-4 OR 124-38-9 OR 3347-22-6 OR 1593-77-7 OR 2439-10-3 OR 119791-41-2 OR 66230-04-4 OR 283594-90-1 OR 203313-25-1 OR 16672-87-0 OR 74-85-1 OR 80844-07-1 OR 26225-79-6 OR 153233-91-1 OR 97-53-0 OR 58073-76-0 OR 50-33-9 OR 126833-17-8 OR 13684-63-4 OR 113158-40-0 OR 517875-34-2 OR 473798-59-3 OR 134098-61-6 OR 67306-00-7) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

(38421-90-8 OR 38363-29-0 OR 37338-40-2 OR 28079-04-1 OR 16974-11-1 OR 54364-62-4 OR 33189-72-9 OR 16725-53-4 OR 20711-10-8 OR 31654-77-0 OR 53042-79-8 OR 34010-21-4 OR 86252-74-6 OR 56578-18-8 OR 40642-40-8 OR 33956-49-9 OR 112-72-1 OR 56683-54-6 OR 28079-04-1 OR 112-66-3 OR 16974-11-1 OR 112-66-3 OR 54364-62-4 OR 54364-63-5 OR 50933-33-0 OR 53042-79-8 OR 56219-04-6 OR 58594-45-9 OR 38421-90-8 OR 38421-90-8 OR 37338-40-2 OR 38363-29-0 OR 28079-04-1 OR 28079-04-1) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

(53939-28-9 OR 34010-21-4 OR 112-53-8 OR 16974-11-1 OR 33956-49-9 OR 638-59-5 OR 163041-94-9 OR 163041-87-0 OR 104040-78-0 OR 158062-67-0 OR 145701-23-1 OR 1390661-72-9 OR 79622-59-6 OR 83066-88-0 OR 272451-65-7 OR 131341-86-1 OR 142459-58-3 OR 62924-70-3 OR 103361-09-7 OR 2164-17-2 OR 239110-15-7 OR 658066-35-4 OR 2699-79-8 OR 361377-29-9 OR 951659-40-8 OR 61213-25-0 OR 69377-81-7 OR 958647-10-4 OR 66332-96-5 OR 907204-31-3 OR 133-07-3 OR 173159-57-4) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

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[EU Pesticides Database - Active substances \(europa.eu\)](http://europa.eu)

(33189-72-9 OR 56578-18-8 OR 38421-90-8 OR 38363-29-0 OR 33956-49-9 OR 53880-51-6 OR 55774-32-8 OR 56683-54-6 OR 34010-21-4 OR 53939-28-9 OR 20711-10-8 OR 58594-45-9 OR 65128-96-3 OR 14959-86-5 OR 40642-40-8 OR 28079-04-1 OR 64470-32-2 OR 35835-80-4 OR 16974-11-1 OR 56219-04-6 OR 35153-15-2 OR 16725-53-4 OR 53042-79-8 OR 31654-77-0 OR 53120-27-7 OR 52207-99-5 OR 571-58-4 OR 112-30-1 OR 86-86-2 OR 86-87-3 OR 3100-04-7 OR 2905-69-3 OR 90-43-7 OR 78821-43-9 OR 1214-39-7 OR 148-24-3) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

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(83164-33-4 OR 50563-36-5 OR 163515-14-8 OR 110488-70-5 OR 13708-85-5 OR 3347-22-6 OR 112-53-8 OR 112-66-3 OR 1593-77-7 OR 2439-10-3 OR 119791-41-2 OR 66230-04-4 OR 16672-87-0 OR 26225-79-6 OR 74-85-1 OR 80844-07-1 OR 153233-91-1 OR 97-53-0 OR 112-05-0 OR 120928-09-8 OR 126833-17-8 OR 113158-40-0 OR 517875-34-2 OR 67306-00-7 OR 473798-59-3 OR 134098-61-6 OR 10045-86-0 OR 10058-44-3 OR 104040-78-0 OR 158062-67-0 OR 145701-23-1 OR 1390661-72-9 OR 83066-88-0 OR 79622-59-6) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(272451-65-7 OR 131341-86-1 OR 142459-58-3 OR 62924-70-3 OR 103361-09-7 OR 2164-17-2 OR 239110-15-7 OR 658066-35-4 OR 361377-29-9 OR 951659-40-8 OR 61213-25-0 OR 69377-81-7 OR 958647-10-4 OR 66332-96-5 OR 907204-31-3 OR 133-07-3 OR 173159-57-4 OR 68157-60-8 OR 22259-30-9 OR 15845-66-6 OR 98886-44-3 OR 57-48-7 OR 76703-62-3 OR 106-24-1 OR 77-06-5 OR 83313-40-0 OR 1071-83-6 OR 943831-98-9 OR 100784-20-1 OR 870721-81-6 OR 629-70-9 OR 78587-05-0 OR 7722-84-1 OR 10004-44-1) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(35554-44-0 OR 114311-32-9 OR 1511720-78-7 OR 144550-36-7 OR 140923-17-7 OR 7720-78-7 OR 875915-78-9 OR 82558-50-7 OR 141112-29-0 OR 7631-86-9 OR 143390-89-0 OR 50-81-7 OR 52-90-4 OR 9008-22-4 OR 23960-07-8 OR 8002-43-5 OR 2164-08-1 OR 94-74-6 OR 94-81-5 OR 12057-74-8 OR 121-75-5 OR 123-33-1 OR 9050-36-6 OR 173662-97-0 OR 374726-62-2 OR 16484-77-8 OR 1417782-03-6 OR 110235-47-7 OR 15302-91-7 OR 131-72-6 OR 400852-66-6 OR 104206-82-8 OR 139970-56-2 OR 57837-19-1 OR 70630-17-0) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(108-62-3 OR 144-54-7 OR 137-42-8 OR 137-41-7 OR 41394-05-2 OR 67129-08-2 OR 125116-23-6 OR 161050-58-4 OR 9006-42-2 OR 3060-89-7 OR 220899-03-6 OR 21087-64-9 OR 74223-64-6 OR 51596-10-2 OR 15299-99-7 OR 111991-09-4 OR 23135-22-0 OR 1003318-67-9 OR 42874-03-3 OR 76738-62-0 OR 64742-46-7 OR 72623-86-0 OR 8042-47-5 OR 97862-82-3 OR 112-05-0 OR 66246-88-6 OR 40487-42-1 OR 494793-67-8 OR 219714-96-2 OR 183675-82-3 OR 106700-29-2 OR 13684-63-4 OR 7803-51-2 OR 1918-02-1) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR

nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(137641-05-5 OR 243973-20-8 OR 23103-98-2 OR 29232-93-7 OR 298-14-6 OR 41607-57-2 OR 88805-35-0 OR 24579-73-5 OR 111479-05-1 OR 145026-81-9 OR 23950-58-5 OR 189278-12-4 OR 52888-80-9 OR 94125-34-5 OR 178928-70-6 OR 175013-18-0 OR 129630-19-9 OR 8003-34-7 OR 96489-71-3 OR 179101-81-6 OR 55512-33-9 OR 53112-28-0 OR 688046-61-9 OR 95737-68-1 OR 422556-08-9 OR 7631-86-9 OR 90717-03-6 OR 94051-08-8 OR 100646-51-3 OR 119738-06-6 OR 64309-03-1 OR 122931-48-0 OR 87392-12-9 OR 21293-29-8) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(874967-67-6 OR 175217-20-6 OR 130561-48-7 OR 67233-85-6 OR 7647-14-5 OR 144-55-8 OR 824-39-5 OR 824-78-2 OR 99573-82-7 OR 935545-74-7 OR 168316-95-8 OR 283594-90-1 OR 203313-25-1 OR 118134-30-8 OR 57-50-1 OR 99105-77-8 OR 141776-32-1 OR 946578-00-3 OR 2699-79-8 OR 231-722-6 OR 14807-96-6 OR 107534-96-3 OR 112410-23-8 OR 119168-77-3 OR 79538-32-2 OR 335104-84-2 OR 5915-41-3 OR 112281-77-3 OR 112-72-1 OR 148-79-8 OR 317815-83-1 OR 79277-27-3 OR 89-83-8 OR 57018-04-9) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(2303-17-5 OR 7758-98-7 OR 106040-48-6 OR 55335-06-3 OR 141517-21-7 OR 135990-29-3 OR 143294-89-7 OR 131983-72-7 OR 142469-14-5 OR 57-13-6 OR 283159-90-0 OR 53120-27-7 OR 1314-84-7 OR 137-30-4 OR 156052-68-5 OR 91465-08-6 OR 102-76-1 OR 102851-06-9) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

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(7440-42-8 OR 7440-48-4 OR 7440-50-8 OR 7439-89-6 OR 7439-96-5 OR 7439-98-7 OR 7440-66-6 OR 150-39-0 OR 20846-91-7 OR 67-43-6 OR 85120-53-2 OR 641632-90-8 OR 1170-02-1 OR 641633-41-2 OR 475475-49-1 OR 60-00-4 OR 23351-51-1 OR 131669-35-7 OR 8062-15-5 OR 35998-29-9 OR 461-58-5 OR 94317-64-3) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel) AND (crop OR farm OR toxicology OR ecotoxicology OR release OR pest OR plant growth OR plant nutrition)

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(87-69-4 OR 30507-70-1 OR 210880-92-5 OR 52315-07-8 OR 86479-06-3 OR 60207-90-1 OR 26046-85-5 OR 51-03-6 OR 35691-65-7 OR 2682-20-4 OR 26530-20-1 OR 55406-53-6 OR 64359-81-5 OR 122453-73-0 OR 3380-30-1 OR 67375-30-8 OR 72963-72-5 OR 135410-20-7 OR 64-19-7 OR 107-02-8 OR 7778-54-3 OR 7782-50-5 OR 7681-52-9 OR 68424-85-1 OR 15879-93-3 OR 20859-73-8 OR 139734-65-9 OR 50-81-7 OR 131860-33-8 OR 143447-72-7 OR 12069-69-1 OR 22781-23-3 OR 1302-78-9 OR 65-85-0 OR 90-43-7 OR 14915-37-8) AND ((nanomaterial OR nanoform OR nanoformulation OR

nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(10043-35-3 OR 56073-10-0 OR 28772-56-7 OR 79-08-3 OR 1305-62-0 OR 37247-91-9 OR 39445-23-3 OR 1305-78-8 OR 10605-21-7 OR 124-38-9 OR 59-50-7 OR 3691-35-8 OR 67-97-0 OR 27519-02-4 OR 77-92-9 OR 106-23-0 OR 61789-18-2 OR 1317-38-0 OR 7440-50-8 OR 20427-59-2 OR 7758-99-8 OR 1111-67-7 OR 5836-29-3 OR 8001-58-9 OR 312600-89-8 OR 57-48-7 OR 894406-76-9 OR 334-48-5 OR 52918-63-5 OR 731-27-1 OR 1317-39-1 OR 7173-51-5 OR 56073-07-5 OR 104653-34-1 OR 35367-38-5 OR 165252-70-0 OR 12179-04-3) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(1065124-65-3 OR 52304-36-6 OR 80844-07-1 OR 120068-37-3 OR 90035-08-8 OR 131341-86-1 OR 50-00-0 OR 111-30-8 OR 8028-66-8 OR 7647-01-0 OR 74-90-8 OR 7722-84-1 OR 138261-41-3 OR 144171-61-9 OR 7553-56-2 OR 7720-78-7 OR 66603-10-9 OR 79-33-4 OR 50-21-5 OR 91465-08-6 OR 143-07-7 OR 8000-28-0 OR 8001-26-1 OR 12057-74-8 OR 84696-25-3 OR 2527-66-4 OR 86347-14-0 OR 112-12-9 OR 240494-71-7 OR 55965-84-9 OR 134-62-3 OR 1085-98-9 OR 133-07-3 OR 66215-27-8 OR 7727-37-9 OR 112-05-0) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

(3391-86-4 OR 124-07-2 OR 494793-67-8 OR 8006-90-4 OR 79-21-0 OR 52645-53-1 OR 1802181-67-4 OR 27083-27-8 OR 25655-41-8 OR 24634-61-5 OR 71-23-8 OR 67-63-0 OR 79-09-4 OR 95737-68-1 OR 68909-20-6 OR 65733-16-6 OR 68876-77-7 OR 119515-38-7 OR 61790-53-2 OR 7631-86-9 OR 127-09-3 OR 532-32-1 OR 168316-95-8 OR 7446-09-5 OR 2699-79-8 OR 107534-96-3 OR 533-74-4 OR 153719-23-4 OR 122454-29-9 OR 118712-89-3 OR 8028-52-2 OR 81-81-2 OR 12122-67-7 OR 39515-40-7 OR 68359-37-5) AND (nanomaterial OR nanoform OR nanoformulation OR nanoparticle OR nanodelivery OR nanocapsule OR nanocarrier OR nanocomposite OR nanosphere OR nanostructure OR nanosensor OR nanomicelle OR nanofilm OR nanogel)

8.2 Annex II

Annex II - Summarizing table.xlsx

This file constitute the repository that fully includes all documents deemed to be relevant and, consequently, the extracted data in this Summarising Table. The working team used this table as a guide to create this report. There are five separate Excel sheets in the Excel file:

- i. Relevant, which includes all original publications deemed relevant as well as the data taken from each study separately,
- ii. Review, which includes all pertinent articles categorised as reviews but from which no data extraction was done in agreement with ECHA;
- iii. Grey, which includes any document deemed pertinent and discovered via the grey literature search following the guidelines outlined in section 2.1.3;
- iv. Patent, which includes all registered patents deemed relevant but from which no data extraction was done in accordance with the ECHA agreement;
- v. Others, which is a collection of documents (books, editorials, etc.) that don't fit into any of the other categories.

Columns M through R in the Excel sheet devoted to the original papers deemed relevant comprise the extracted data. It turned out that managing and making use of the massive amount of gathered data required a homogenization of the extracted data.

The data can be filtered in column M based on the constituent materials of the nanoparticle. When more than one material was utilised to create the nanomaterial, those instances are stated using the letter "and" to separate them.

If the active ingredient is present and distinct from the nanoparticle's component materials, its name is listed in column N. Similar to column M, all reported active substances are sorted alphabetically and separated by "and" if more than one is utilised.

In contrast, column O reports the morphology and dimensions of the nanoparticles, using commas to separate information about the same nanoparticle. Instead, data pertaining to various particles is divided into dots. The single number, presented minus the standard deviation, indicates the average; two numbers separated by a "-" indicate the range of nanoparticle sizes. Once more, if there are several pieces of information, they are presented from smallest to largest in ascending order of size.

Lastly, column P displays the nanoparticle's intended use. Once more, if a formulation has many uses, these are listed in alphabetical order with a "/" between each application. A "/" closes the corresponding cell when a data element is absent or undefined.