2

Review of literature

2.1 Background

Agriculture not only feeds people but is the economical backbone of most developing nations. Disastrous changes in the atmosphere and also the growing population worldwide have drastically changed the traditional farming practices [83]. Agriculture methods in general comprise conventional, integrated, and organic farming strategies [84, 85]. In general, a massive amount of synthetic chemicals, pesticides, and weedicides are employed in conventional farming practices to increase crop yield which causes environmental problems such as soil toxicity and loss of soil fertility, *etc* [86]. Extensive use of pesticides causes a series of environmental problems, suchas pathogen and pest resistance, non-point pollution, eutrophication in the water body, soil degradation, bioaccumulation of xenobiotics in the food chain, and loss of biodiversity, etc [87]. The solvents and toxic ingredients of pesticides result in not only serious soil and water pollution but also increase the toxicity in crops and food products through bioaccumulation and eventually become a potential threat to human health [87, 88]. As per The United Nation Environmental Program, a number of pesticides are considered as Persistent Organic Pollutants (POPs) [89]. Such POPs are readily gets absorbed in fatty tissues of animals through food chain due to the phenomena known as bioaccumulation and these chemicals can be found in living organisms far away from their source due to anthropogenic activities [89]. Therefore, developing new eco-friendly and result-oriented strategies in the agricultural sector for more yield to feed the growing population offers novel approaches in a limited time. It is the need of the hour to modernize agricultural sector by accelerating the productivity and nanotechnology with its recent development may be considered as a potential tool to achieve this goal. Nano-products can provide new and better solutions for various agricultural problems such as detection and mitigation of infection long before symptoms are visually evident, nutrient deficiency, or other health problems; delivery of nano-formulated pesticides; delivery of fertilizers; to reduce nutrients loss into the soil by leaching; nano-herbicide for controlling weeds population effectively and their post-implementation detoxification; field sensing systems to monitor the environmental stresses and crop condition to improve plant health against environmental stresses and diseases, *etc* [90]. Such nano-based agricultural technologies are collectively known as nanofarming (**Fig. 2.1**). Therefore, considering the disadvantages of traditional and integrated agricultural methodology, nanofarming strategies may be proposed for revolutionizing the agricultural sector in the future. Recent advances in nanofarming have also demonstrated their efficiency and broad potential relevance in the agricultural sector by assessing environmental factors for optimum use of fertilizers and pesticides for better yield [91]. As a result, various nano-products such as metal NPs, carbon nanotubes, quantum dots, and nano- fibers, etc. are relentlessly gaining importance and applied in agriculture not only as growth promoters, pesticides, insecticides, anti-microbial agents, nano-fertilizers due to non-toxic and environmentally benign nature but also as biosensors for assessing soil quality. These presently developed methods are competent in performing both asa preventive and cautioning device and are also capable of recognizing health-related issues associated with plants [92].

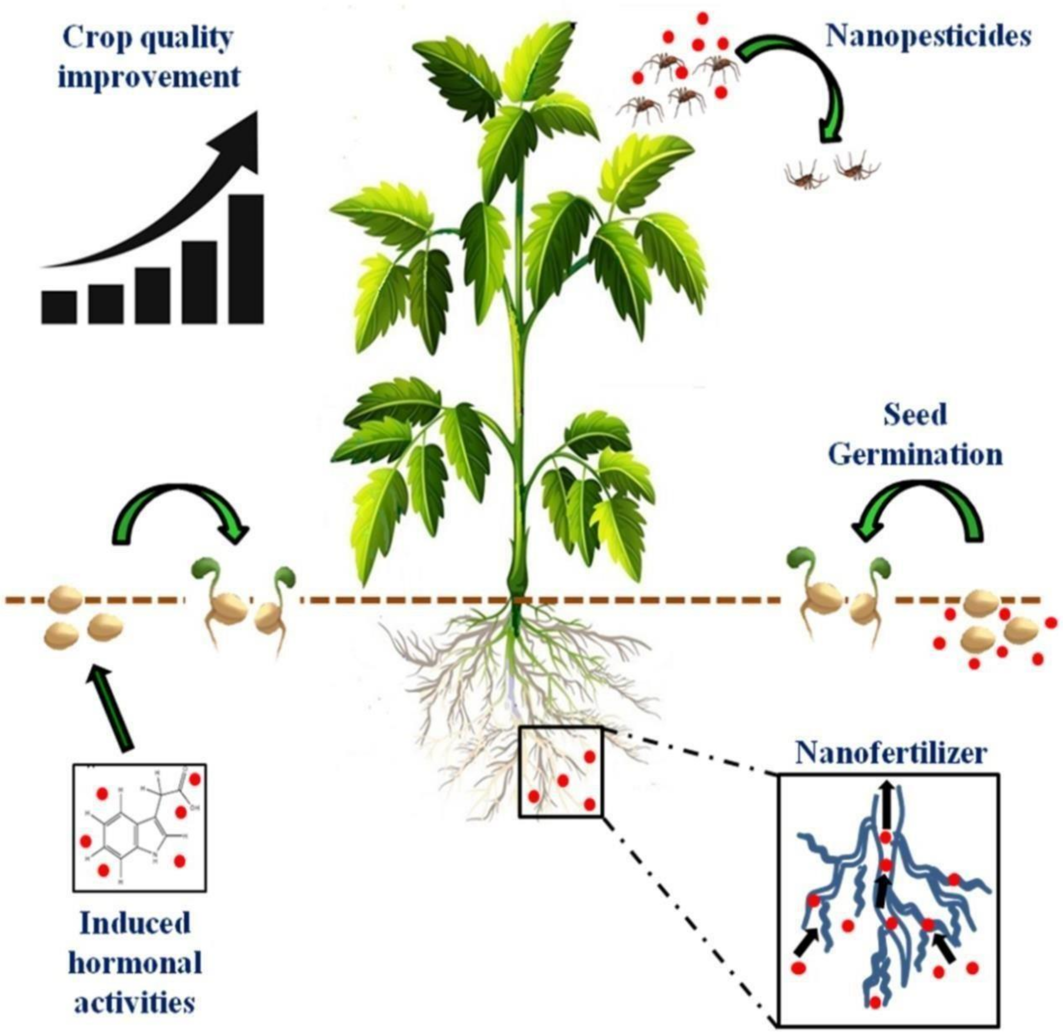

Fig. 2.1 Diagrammatic representation of the scopes of nano-farming which includes the application of nanoparticles (NPs) for inducing and catalyzing hormonal activities for rapid plant growth, improving crop quality and productivity, in the form of nano-pesticides, enhancing seed germination, and as nano-fertilizer

2.2 Mode of synthesis of NPs

Nanomaterials are defined as the materials or particles within a size dimension of 1–100 nm [3]. Conventionally, NPs are synthesized applying two strategies: the top- down approach and bottom-up approach [93]. In the top-down approach, the larger materials are progressively broken down into minute nano-sized materials; whereas,in the bottom-up method, atoms or molecules are arranged into molecular structuresin the nano-meter range [3]. Even though, there are varieties of physical and chemical methods used for NPs synthesis but a majority of the existing techniques are not cost-effective; produce toxic waste, and unstable in nano-form with reduced targetedaction [3]. Hence, nowadays green synthesis emerges as a new eco-friendly and cost- effective method of synthesis of NPs. Green methods are eco-friendly using non-toxic biological agents such as plant leaves, roots, stems, fruits, seeds, metabolites, microorganisms, biosurfactants, etc., and are cost-effective [82, 94 − 98]. The NPs produced are highly stable because the reducing agent itself acts as a stabilizing agent most of the time [94, 99]. Anthocyanins, isoflavonoids, flavonols, chalcones, flavones, and flavanones, etc. in plants; biopolymers such as biosurfactants, exopolysaccharides (EPS), and proteins produced by microorganisms; animal proteins such as saliva, and egg albumin, etc. can reduce metal ions into metal NPs [99]. The mode of synthesis of various metal NPs is shown in **Table 2.1**.

Table 2.1 Antimicrobial properties of different metal NPs and their mode of synthesis

Name of metal NPs	Reducing agent	Target organism	References
Au NPs	*Trichoderma viride* and *Hypocrea lixii*	*Pseudomonas syringae, Escherichia coli,* and *Shigella sonnei*	[100]
Au NPs	Blue green alga	*Bacillus subtilis* and *Staphylococcus aureus*	[47]
Au NPs	Sulfite reductase purified from *E. coli*	*Aspergillus fumigates, Fusarium oxysporum, Trichoderma parceramosum* and *Candida albicans*	[59]
Au and Ag NPs	Corn extract	*E. coli, P. aeruginosa and S. aureus*	[60]
Ag NPs	*Melaleuca alternifolia* leaf extract	*S. aureus, MRSA, S. epidermidis, S. pyogenes, K. pneumoniae, P. aeruginosa, Trichophyton mentagrophytes, C. albicans,* herpes simplex virus type 1 (HSV-1), and	[15]

		herpes simplex virus type 2 (HSV-2)	
Ag NPs	*Carya illinoinensis* leaf extract	*E. coli*, *P. aeruginosa*, *S. aureus* and *Listeria monocytogenes*	[101]
Ag NPs	Castor extract	*S. aureus and P. aeruginosa*	[14]
Ag NPs	Banana peel extract	*S. aureus, B. subtilis, P. aeroginusa, E. coli, and C. Albicans*	[102]
Ag NPs	Amino acids	*L. monocytogenes* and *E. Coli*	[58]
Ag and Au NPs	*Piper nigrum* leaf extract	*Aeromonas liquefaciens, Enterococcus faecalis, Klebsiella pneumonia, Micrococcus luteus, Salmonella typhimurium, Vibrio cholerae*, *C. albicans*, *Cryptococcus* sp., *Microsporum canis, Trichophyton rubrum*	[103]
Ag NPs	Fluorescent light mediated synthesis using the protein extract of weaver ant larva	*E. coli* and *S. aureus*	[104]
Ag NPs	Lipopeptide biosurfactant	*P. aeruginosa* and *B. subtilis*	[105]
CuO NPs	Chemical synthesis by reverse micelle method	*K. pneumoniae, S. typhimurium, E. aerogenes*	[63]
ZnO NPs	Hydrothermal approach	*E. coli*	[13]
ZnO NPs	*Vitex trifolia* L leaf extract	*S. aureus, B. subtilis, P. aeruginosa, P. mirabilis,* and *E. coli*	[106]
CuO NPs	Actinomycetes	*S. aureus, B. cereus, Proteus mirabilis, Edwardsiella tarda, Aeromonas caviae, Aeromonas hydrophila* and *Vibrio anguillarum*	[54]

2.3 Applications of NPs in agriculture

The contribution of smart nanotechnology to sustainable farming is a chief need to improve traditional farming practices into modern smart practices [107]. The use of new-generation fertilizers and pesticides having nano-formulation and nano-encapsulation provides the site-specific target and controlled delivery of active ingredients to the plants [108]. The growth of nano-fertilizer, nano-pesticides, nano-herbicide, and nano-sensor as modern smart delivery systems has opened up a new form of sustainable applications in various sectors of agriculture [109]. Diverse types of nanomaterials, their oxides, and nano-formulations have shown promising results in enhancing the seed germination rate, and plant growth with better yield [107, 110]. The controlled and site-specific delivery of active ingredients of farming input helpsin providing sustainable results in reducing environmental pollution, and the numberof expenses on farmers for the use of traditional fertilizers and pesticides [110].

2.3.1 Nanofertilizers

Nano-fertilizers are nanomaterials encapsulating nutrients for the smart delivery of nutrients to fulfill the nutrient necessities of plants [111]. Due to nano-products having high reactive activity, they interact with fertilizers resulting in efficient uptake of nutritional elements in plants through both roots and leaves [91, 112]. They are divided into three categories based on the nutrient requirement of plants, viz., macronutrient nano-fertilizer, micronutrient nano-fertilizer, and nano-particulatefertilizer.

2.3.2 Macronutrient nanofertilizers

Nano-fertilizers are composed of nitrogen (N), calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) which are required in the highest amounts for plant productivity. Nano-fertilizers reduce the high amount of nutrient requirement due to their high-volume-to-surface ratio and increase the success rate as compared to traditional fertilizers and such macronutrient nano-fertilizers have shown encouraging results even in field studies [107]. Nano-fertilizers have the advantages over traditional fertilizers as they may increase the use of the nutrients by the plants by minimizing their escape into the ground water [113].

2.3.3 Micronutrient nanofertilizer

Micronutrients are trace elements which include Au, Ca, Ce, CNTs, Cu, Fe/Si, Mg, Mn, Mo, P, Ti, Zn, etc. required in small quantity (≤ 100 ppm) but plays a vital role in various metabolic processes in plants [107]. These micronutrients in nano-form improve their bioavailability to the plants and show a major improvement in plant growth and nutritional quality [107].

2.3.4 Nanoparticulate fertilizer

Some NPs like TiO_2, SiO_2 and carbon nano-tubes, etc. have also shown plant growth-promoting activity [107]. For instance, a mixture containing TiO_2 and SiO_2 NPs has increased nitrogen fixation in *Glycine max* and improved its seed germination rate and plant growth by increasing nitrate reductase activity in roots and leaves leading to enhanced water-absorbing and utilizing abilities; stimulated antioxidant system, and finally improved its resistance to adverse conditions [114]. TiO_2 NPs were also reported to be increasing total nitrogen, protein, and chlorophyll content in *Spinacia oleracea* by inducing Rubisco and Rubisco activated complex to promote Rubisco carboxylation for enhancing photosynthetic rate [115].

2.3.5 Nanopesticides

Formulation of nano-pesticides using metal NPs is a highly challenging subject in the pesticide industry. It is beneficial for the smart and controlled release of active pure elements by changing the outer coating material which usually releases a low dose of the compound during an extended period and as a result, it decreases the excessive use of chemical pesticides [116]. Some of the metals and its oxide in nano-form such as ZnO, TiO_2, CuO and SiO_2, *etc*. are extensively used as pesticides and fungicides to shield the plant from numerous bacterial diseases and also control the increase of microbial activity [107]. Not only human but also plant pathogens can be controlled and prevented by using NPs. Nanoparticles also reduce the growth of conidiophores and conidia of fungi which results in the decrease of fungal hyphae [117, 118]. Studies have shown TiO_2 NPs with better efficiency against litchi fungal disease as compared to conventional fungicides [119]. Although drug and DNA delivery inanimal cells and tissue is extensively done by using Si NPs till now, least explored in the case of plants due to the presence of a cell wall that inhibits its delivery into the system. However, the end cap of Au NPs when combined with mesoporous silica made it capable of delivering chemicals, protein, and other necessary nutrients injected inside the plant using a gene gun under controlled state research [120]. An additional benefit of nano-products for the protection of plants is smart site-targeted delivery of necessary elements [108]. Nano-formulations in water and oil with increased solubility of the desired pesticide against different toxic and harmful pests show better efficiency over traditional pesticides [121, 122]. Ag NPs are one of the most widely reported metal NPs which show potential antimicrobial activities against several plant bacterial pathogens such as *Biploaris sorokinniana, Botrytis cinerea, Colletotrichum gloeosporioides, Fusarium culmorum, Phythium ultimum, Phoma sp., Megnaporthe grisea, Trichoderma sp., Sphaerotheca pannasa*, and *Rhizoctonia solani*etc. [123, 124]. Additionally, Si NPs when used by researchers in combination with silver revealed 100% effectiveness against cucurbits disease, i.e., powdery mildew disease [125]. They have also been used for smart delivery of desired pesticides whichshowed their potential as a substitute for a widely used fungicidal antibiotic validamycin [122]. Metal NPs have shown their antimicrobial efficacy against both Gram-positive and negative plant pathogens such as *Xanthomonas oryzae*, *Xanthomonas campestris*, etc., and also some of the fungi such as *Fusarium sp.*, and *Phytopthora infestance, etc*. [126], [127]. Some of the widely reported NPs against various bacterial and fungal pathogens are shown in **Table 2.1**.

2.4 Role of NPs in seed germination

In recent years, many researchers have confirmed that metal NPs have great importance in agricultural growth and enhancement [98, 128]. In industrial farming, fast and consistent germination of seed and seedling emergence plays a significant role in achieving a successful yield. Germination starts with water uptake in the mature dry seeds (known as imbibition) and ends with the elongation of the sprouting axis which forms the root and shoots. Since mature seeds are quite dry and hence needa sufficient quantity of water to begin the cellular functions and growth [21]. Thus, metal NPs promote easy uptake of water and nutrient required for proper germination by penetrating the seeds [4]. Studies have demonstrated higher uptake of water in nanoprimed seeds of *Zea mays*, *Glycine max*, and *Cicer arietinum* leading to higher germination rate (90%) and enhanced plant growth as compared to non-treated seeds (30%) [98]. The α-amylase is an important enzyme that is involved in starch hydrolysis and is synthesized de novo during seed germination (**Fig. 2.2a**). Thus, the enrichment of α-amylase activity during germination of the seed is significant to support plant growth and it is hypothesized that metal NPs can catalyze the activity of α-amylase by entering through the cellular pores of the seeds by taking the advantage of their nano-size and as a result enhances the seed germination rate (**Fig. 2.2b**) [21]. NPs may penetrate seed coat by creating small pores, increasing water uptake and up- regulation of the expression of aquaporin and Reactive Oxygen Species (ROS) genes involved in water uptake. Besides, root vitality in terms of their ability to take up nutrients and water, and level of metabolic activity are governed by theirdehydrogenase activity. Metal NPs can significantly improve the root dehydrogenase activity, thus enhancing the water absorption capability by the seedlings [36, 129]. Based on scientific evidence, it may be said that both ROS and aquaporins participate in improving the germination of seeds (**Fig. 2.2b**). Metal NPs also show antioxidant potential which prevents oxidative destruction of lipids and maintains the structural and functional integrity of cells. Superoxide dismutase (SOD) is one of the enzymes in the combination of catalase known for its involvement in enzymatic oxidant detoxification. Nano-priming treatment of seeds may stimulate the SOD and other reactive oxygen species having scavenging activity in seeds that helps to protect the cell from the damage caused by free radicals [38]. For *e.g.*, TiO_2 NPs have shown an increase in the growth rate by enhancing the seed stress resistance and promoting capsule penetration for the uptake of water and oxygen required for rapid germination [130]. A case study on the role of various metal NPs in plant growth enhancement is shown in **Table 2.2**.

Table 2.2 Case study of various metal NPs in plant growth augmentation

Name of NPs	**Target crop species**	**Mode of agricultural application**	**Average size of NPs synthesized (nm)**	**Shape**	**Duration of treatment**	**References**
TiO_2 NPs	Fennel (*Foeniculum vulgare* Mill)	Seed germination	21	Tetragonal	14 days	[131]
Ag NPs	Corn (*Zea mays* L.), Watermelon (*Citrullus lanatus*) and Zucchini (*Cucurbita pepo* L.)	Seed germination	20	Nano-powder	2 h	[30]
Ag NPs	Rice seeds	Seed germination and starch metabolic process	11.2	Spherical	24 h	[21]
Multi-walled carbon nanotubes	Wheat (*Triticum aestivum*)	Seed germination, root elongation, stem length, and vegetative biomass	100-5500 50-630	Tube Bundles	7 days	[36]
Au NPs	*Brassica juncea*	Growth and Seed yield	10 - 20	Spherical	5 days	[32]
Au NPs	Rice, Radish, Pumpkin, and Perennial ryegrass	Seed germination	-	Monolayer	5 days	[33]
Au NPs	*Arabidopsis thaliana*	Seed germination	-	Spherical	5 days	[132]

ZnO and FeO NPs	Wheat	Plant growth and cadmium (Cd) accumulation	20-30 50-100	-	24 h	[34]
ZnO and CuO NPs	Wheat	Seed germination	-	-	4 h	[31]
ZnO and TiO_2 NPs	*Cicer arietinum* L	Seed germination	-	-	12 days	[133]
ZnO NPs	*Oryza sativa* L	Seed germination	-	-	1 h	[134]
CuO NPs	Cauliflower and tomato plants	Growth, metabolism and antioxidant activity	20-30	Spherical	24 h	[135]
Citrate-coated magnetite nanoparticles	Wheat	Seed germination, root and shoot lengths, and heavy metal accumulation	10-20, 2-10, <40	Cubic, spherical, and rod-shaped	7 days	[137]
ZnO, TiO_2, CuO, Ag	Oat and Berseem	Seed germination and Yield	-	-	10 min	[138]
ZnO	Chilli (*Capsicum annuum* L.)	Seed germination and Seed Vigour	-	-	6 h	[139]
MgO	Peanut (*Arachis*	Seed germination	0.4291	Cubic	12 h	[140]

	hypogaea L.)					
$Mg(OH)_2$ NPs	*Z. mays* seed	Seed germination	50-100	Crystalline	7 days	[141]
Al_2O_3	Tomato (*Solanum lycopersicum* L.)	Growth, Physiology and Yield	28	Crystalline	2 days	[142]
CuO			18			
TiO_2 and			4.6			
ZnO NPs			34			
Polystyrene nanoplastics (PSNPs)	Wheat (*Triticum aestivum* L.)	Seed germination and seedling growth	-	-	2 days	[143]
Fe_2O_3 NPs	Watermelon Seedlings	Seed priming	19−30	Low-crystalline or amorphous form	14 h	[144]

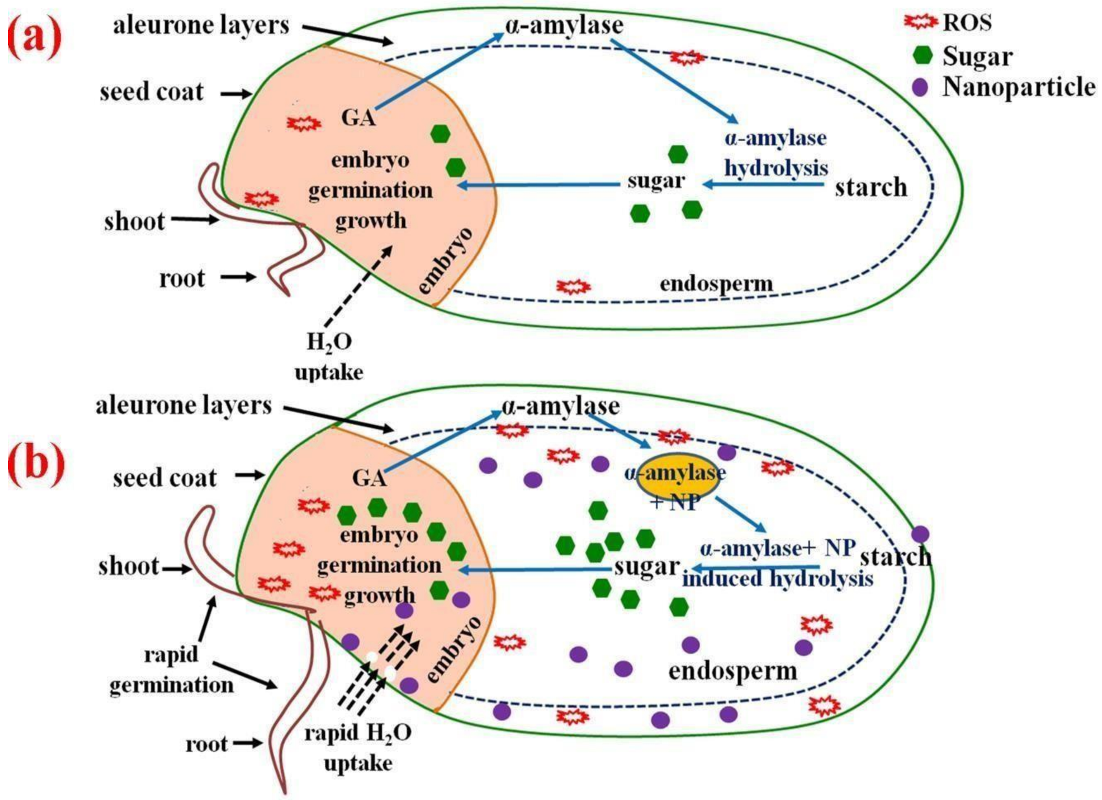

Fig. 2.2 Hypothetical depiction of nano-primed seed germination (adapted from [21] under Creative Commons Attribution 4.0 International License). (a) Slow seed germination and growth rate (without nano-priming) caused due to low metabolic rate because of slow uptake of water. (b) Enhanced seed germination in nano-primed seed due to the rapid uptake of water as NPs penetrate the seed coat causing rapid water uptake

2.5 Mode of uptake of NPs in plants and their accumulation

Plants are a vital part of the atmosphere and therefore transportation of NPs through the environment into the plants is a significant aspect of estimating the effect of nanomaterials on the metabolism of the plant [145]. The concepts regarding uptake, bio-accumulation, translocation, and transmission of the NPs in the plant body are stillnot clear [146]. The uptake and internalization of NPs in plants is an active event that may depend on different exposure circumstances, chemical properties of NPs, their size, surface property, aggregation pattern, movement, and interactions with ecosystems and type of crop species [108, 147]. The main location for contact and reaction during the entry of the NPs is the cell wall of plant cells, the process for which is still not well understood [146]. Plants absorb minerals non-selectively and during the process, some metal ions may get absorbed. Following absorption, NPs subsequently get translocated and finally becomes accumulated in various plant parts forming complexes with carrier proteins. Particular NPs are selected by a specific plant but reject other NPs the reason behind which remains unclear [148]. Inside the cells, NPs may directly alter membranes, cell structures, cell molecules, and also other protective mechanisms [146]. The cell walls present in plant cell acts as a preventive barrier to pass various elements larger than the size of the microscopic pores present on it and hence the NPs smaller than the size of the microscopic pores can easily pass through. Nanoparticles also use stomata and the base of root hairs for entry into the leaves' surface, which are then transported to different parts of the plant [108] (**Fig. 2.3**). Nanoparticles that are found larger than the diameter of root hair pores have a tendency to accumulate at the surface but NPs which are smaller get absorbed and transported to other organs of the plant. Some NPs get accumulated in extracellular space, while other NPs remain inside the cells [148]. Nanoparticles, upon release to the environment, may transform by interacting with various other organic and inorganic chemicals and also with other factors such as radiation UV light [149]. Translocation and accumulation of engineered nanomaterials occur differentially inside stems, petioles, leaves, and fruits of various crops [130].

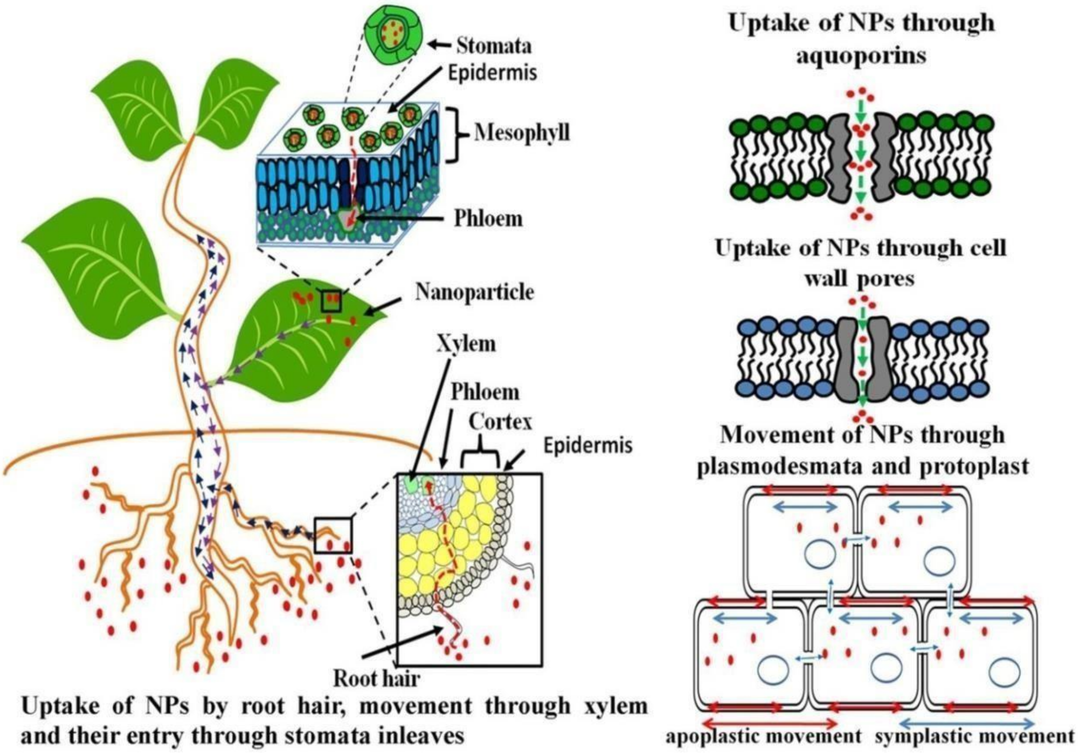

Fig. 2.3 Various mode of uptake of NPs in plants by root hair, their transportation through xylem, aquaporins and plasmodesmata

Nanoparticles can also be combined with some specific, non-specificmembrane transporter proteins or some chemical compounds in root exudates and later, can be transported to the plants [150, 151]. Many metal NPs have shown their capacity to transport into plants through ion transporters [152] (**Fig. 2.3**). Nanoparticles can be accumulated inside the plant root, and shoot tissues as intact particles but some organic molecules such as aldehydes, ketones, terpenoids or alkaloids, etc. released from the root tips transform metal salts into NPs via reduction which subsequently can be transported into the plant [153, 154]. The method of NPs uptake by plants also varies depending on the means of contact. If NPs are applied to foliage, they directly settle down on the aerial parts of plants but if applied to soil, these NPs slowly move in soil pores through various forces, such as water movement and physical pressure, and subsequently enter through the roots [155]. Finally, when NPs come in contact with a root or leaf surface, NPs stick to the plant surfaces via various forces such as electrostatic, hydrophobic, and van der Waals forces [156, 157]. Following this process, wrapping or docking with the surface occurs and subsequently NPs enter the cells near the surface [158]. Uptake of NPs, in general, takes place through the root hairs or by the lateral roots; however, the uptake in leaves takes place through the stomatal opening and trichomes [159]. After entering through the roots and leaves, NPs start traveling from one cell to another and subsequently move upward and downward through xylem or phloem tissues, respectively (**Fig. 2.3**) [158]. Nanoparticles enter through the stomata, trichomes, and cuticle [160] when it is applied on aerial parts and finally reach the phloem which is subsequently transported downward [158]. Similarly, they are absorbed by roots when coming in contact with the soil [161]. The cell–cell movement of NPs occurring through various modes may also show the way to their entry into the xylem tissue. Finally, all the way through the xylem, these NPs are carried upward to different parts of the plant [161]. The structural differences in the xylem may guide a different route of NPs internalization into the plant body. Internalization of NPs may take place by apoplastic or symplastic mode or they may enter into vascular tissues through plasmodesmata (**Fig. 2.3**).Findings have shown that symplastic transport is the most acknowledged pathway for intercellular uptake of NPs known till now but some studies have also explained the apoplastic mode of transport and various mechanisms involving plasmodesmata, carrier proteins, aquaporins, ion channels, and endocytosis. Nanomaterials can disperse into the space sandwiched between the plant cell wall and plasma membrane, commonly known as an apoplastic route [162]. Particles may directly arrive at the endodermis through the apoplast without passing the border of epidermal and cortical cells [163] (**Fig. 2.3**). The symplastic route is a well-regulated path for transporting nanomaterials in the plants which involve uptake of solutes in the epidermis or cortex and transport from cell to cell through plasmodesmata [164] (**Fig. 2.3**).

2.6 Effect of NPs on plant hormones

Plant hormones are active natural substances that are made by a plant's metabolism which regulates many physiological responses during plant growth [165]. An important key to plant toxicity is the content and action of plant hormones. Nanomaterials are known to show a major control over plant hormonal activities [166]. According to some researchers, CeO_2 NPs show no significant effect on indole- 3-acetic acid, abscisic acid, and gibberellic acid (GA) on the leaves of Bt-transgenic and conventional cotton against the control group [167]. On the other hand, the hormonal content of the indole-3-acetic acid and the abscisic acid showed an increase in activities on roots of transgenic and non-transgenic rice species when exposed to Fe_2O_3 nanoparticles (γFe_2O_3) [168]. Some researchers found that concentrations of phytohormone decreased in rice when it was treated with carbon nanotubes [169]. Since silver ions have the capacity in inhibiting the production of ethylene, so the communication between IAA and ethylene significantly weakens and thus the regulation of multiple plant processes gets altered, ranging from seed germination to organ senescence, mostly the role of ethylene as an inducer of fruit ripening [166]. Biosynthesis of auxins and cytokinins gets gradually suppressed with an increase in the concentration of NPs into shoot apical meristems but cytokinin called cis-zeatin gets increased when treated with NPs in roots in response to stress [170]. Some researchers have claimed an increase in the levels of overall cytokinins compared to control pepper plants when exposed to Ag NPs [171]. Research has also confirmed up-regulation of the stress hormone, *i.e.*, abscisic acid with an increase in the concentration of ZnO NPs principally on the leaves and apices compared to salicylic acid where stimulation was seen mostly on the roots and leaves [170].

2.7 Effect of NPs on plant physiological indices

Fundamental physiological indices of plants show the impact of NPs including the germination rates, root elongation rates, biomass, and a large number of leaves [172]. It was explained in a few research works that the accumulation of NPs depends on their concentration and their time of exposure [172]. Nanomaterials have shown both positive and negative impacts on plant physiology as demonstrated by researchers. Metal NPs can boost the action of α-amylase and as an effect enhances seed germination rate [21]. Metal NPs are also capable of significantly developing the root dehydrogenase property, thus enhancing the water absorption capability of the seedlings [36, 129]. The growth and propagation of various plant species may be affected owing to wide-ranging applications of the metal and its oxide nanomaterials and its uptake into the plant. Some studies showed that a low concentration of ZnO and FeO NPs enhanced the growth of shoots, roots, and spikes in wheat [34]. Low concentration of ZnO, TiO_2, FeO, CeO_2, SiO_2 NPs, *etc.* also have shown their role in increasing the weight of shoots and roots of wheat, chickpea, mung bean (*Vigna* *radiata*), rice, sorghum, and tomato, etc. [36, 129, 173]. In contrast, a few pieces literatures have also shown the inhibition of a few plant species on exposure to nanomaterials [174, 175]. For instance, Ag and TiO2 NPs were reported to have reduced the fresh and dry weight of shoot and root in rice and wheat (*Triticum aestivum*) respectively depending on the dose and size of NPs [176]. However, most other research also shows that the nanomaterials have enhanced the total content of chlorophyll and the catalase content and reduced ascorbate peroxidise amount which is an H_2O_2-scavenging enzyme and is indispensable for the protection of chloroplasts and other cell constituents from damage by H_2O_2 and hydroxyl radicals [•OH] inside the plant and thus advocating the futuristic application of nano-farming strategies [177 − 179].

2.8 Effect of NPs on crop quality

The use of nanotechnology for fruit or crop production may cause improvement in the quality, shelf-life, freshness, and health of the plant and its products. Moreover, it can also play a major role in improving plant growth and also decrease the toxic effects of stress conditions [180]. Nanoparticle shows varied effects on crop yield and nutritional quality of various food crops. Although NPs are known to affect the basic physiology of the plant, there is a lack of literature on their role in the quality of crops. It has been hypothesized by some researchers that metal NPs have the potential to penetrate the cellular membrane of the root which may form uneven pores leadingto the absorption and transportation of NPs by the plants at an uneven rate [181]. For example, it has been seen that CeO_2 NPs gives varying effects on the yield and quality of plant crops depending on the type of plant and cultivars [181]. For instance, in a study, the introduction of CeO_2 NPs to the wheat plant has caused better yield, length of the spike, and the number of spikelets. The grain yield of wheat was increased significantly upon application of CeO_2 NPs [181]. The use of ZnO NPs demonstrated an increase in the starch and protein content in cucumber [182]. Similarly, Ag NPs showed an increase in the potassium (K) content in tomato fruits; whereas the same study indicated the reduction of magnesium (Mg), phosphorus (P), and sulphur (S) content [154]. Researchers also demonstrated a significant crop yield up to 60% when used urea-coated hydroxyapatite NPs as compared to pure urea in control under laboratory conditions [183]. Zn NPs at an optimum concentration could be used to enhance the quality of the pigeon pea seeds [184]. In contrast, a few researches have also demonstrated the cytotoxic effect of hydroxyapatite nano- and macro-particles [185, 186]. In addition to this, there are no adequate evidences showing field success to support their immediate implementation in the field. Barley upon the introduction of CeO_2 NPs did not form seeds [181], cucumber production declined [186, 187], and also decreased the Fe, sulfur, prolamin, glutelin, lauric and valeric acids, and starch content in rice grains [188]. Use of Ag NPs, CeO_2, and ZnO NPs were reported to be drastically decreased arginine and histidine contents in maize, cucumber, and soybean plants, respectively [188 - 191]. Hence, it may be stated that treatment with NPs may give varied effects on various types of seeds.

2.9 Phytotoxic effect of NPs

Phytotoxicity, growth, and antioxidant resistance response depend on the type of plant species and its seed. The time interval involved in nano-priming is also an important factor that may induce nano-toxicity. However, data and studies related to the phytotoxicity of NPs on grain yield, development, and quality are limited. Some of the earlier literature has suggested that varying toxic responses in plants can becorrelated with a variety of seed sizes, leaves, and the anatomical structure of the xylem (**Fig. 2.4a**) [172]. For example, a comparative evaluation of the effect of CeO2 NPs on different rice varieties has shown that as compared to the control plant, the content of Ce got enhanced in some treated rice varieties [188]. However, some others reported that exposure to ZnO NPs has shown different responses in the surface area of leaf for both the cultivars as compared to control conditions [192]. Researchers have demonstrated an enhanced germination rate in *Glycine max* seeds under the influence of TiO_2 and SiO_2 NPs [114]. Similarly, carbon nanotubes (CNT) and Al NPs were found to increase the germination rate of tomato seeds and elongation of root lengths in radish and rape seedlings respectively [193, 194]. Such observations are likely due to increased water uptake by seeds in the presence of high concentrations of NPs [108].

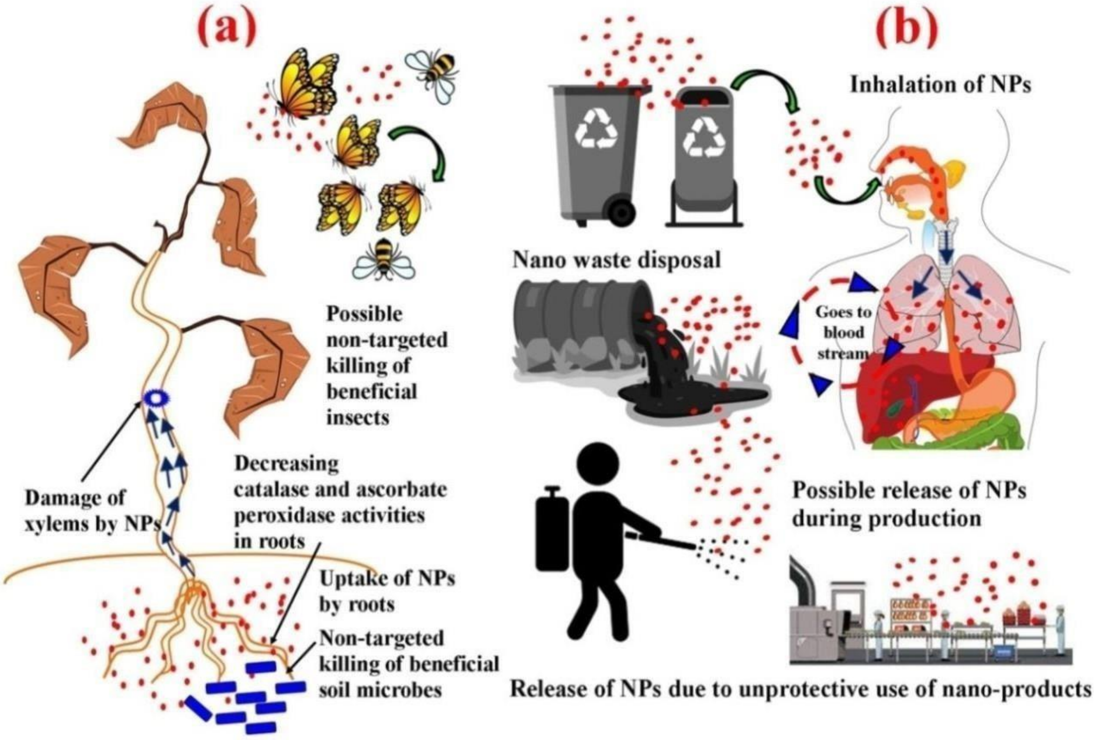

Fig. 2.4 Hypothetical depiction of the possible adverse effect of NPs on (a) plants by damaging the xylems during the uptake through the roots, non-targeted killing of beneficial soil microbes and insects associated with pollination; (b) toxicity in the human body by inhalation during manufacturing, handling, and disposal of NPs

2.10 Factors affecting phytotoxicity by NPs

Recent studies have found that the major factors which stimulate the effects of NPs on plants are the concentration of NPs, size, category, stability; the plant species, doses of NPs as compared to plant seed size, growth medium of the plant, growth stage of the plant, and the coating material of NPs *etc* [166].

2.10.1 Effects of different growth media on NPs phytotoxicity

Nanoparticles have shown different responses to plant growth, depending on specific environmental conditions and growth media used [195]. For example, the biomass of radish showed different effects after treating with CeO_2 NPs depending on the type of sand on which it is grown [156, 157]. The Ce content in the fine and storage roots resulted in higher growth in loamy sand compared to silty loam [156, 157]. Studies also revealed that Ce accumulation is higher in a low organic matter soil than the high organic matter soil when kidney bean plants are treated with CeO_2 NPs [197].

Similarly, different responses of toxicity in plants were caused by CeO_2 NPs in lettuce seeds when treated and incubated with three different types of growth media [198]. Phytotoxicity with Ag NPs gets reduced with rising content of clay and pH whereas it remains unaffected in the content of organic carbon of the soil [199]. It may be noted that aggregation patterns of NPs in solution vary depending on the constituents of the media [200], and hence alters its bioavailability [146]. In addition to the above, the absorption and phytotoxicity rate of NPs by plants may also depend on some of the physical parameters which include the mean diameter and agglomeration behavior of the NPs. Hence, it may be understood that the phytotoxicity of NPs on plants largely depends on specific growth media [195].

2.10.2 Effects of different growth stages on NPs phytotoxicity

The plant shows varying responses to different environmental conditions depending upon their specific growth stages. For example, some evidence showed that basic plant photosynthetic indicators have altered during the entire growth period in the case of cucumber [201]. When plants are exposed to CeO_2 NPs and CuO NPs, the leaf size of emerging seedlings has started decreasing as compared to the control plant, whereas the adult leaves have shown no major differences [201]. According to [197], the activities of catalase and ascorbate peroxidase in roots have revealed a drastic decrease during the 15 days as compared to the 7 days but opposite results were seenin the case of contents of total soluble protein in roots when treated with CeO_2 NP. Hence, it may be understood that better growth rate and yield with no cytotoxicity can be achieved by optimizing the process of nano-priming, choosing specific plant species against specific NPs choosing appropriate growth media, and controlling the size of NPs, *etc*.

2.10.3 Effect of different coating material on NPs phytotoxicity

The effect of NPs on the soil–plant system also depends on the coating of the NPs as coating changes their utility. Nanoparticle-mediated phytotoxicity and solubility vary with the type of coating. Treatment of the soil with Ag NPs has demonstrated an increase in the rate of germination in a variety of species that caused a considerable decrease in the bioavailability and toxicity of silver in the soil. However, very few reports have also demonstrated phytotoxicity by Ag NPs [202]. Since the plant cell wall acts as a protective layer and the pores present in it allow ion exchange, NPs may penetrate the epidermal layers in roots to reach the xylems through which they translocate to the leaves. Therefore, particle size, coating, and surface charge greatly affect the toxicity of NPs [202]. Previous studies reported that increasing the size of particle aggregates would reduce the toxic effect of the metal oxide NPs [194, 200]. Engineered metal oxide NPs may hold significant potential applications in agriculture and gardening, as they may selectively inhibit unwanted plants (such as weeds), kill harmful fungi and bacteria in plant fields, and release essential metal elements for plant growth [108].

2.11 Impact of NPs on the environment and their regulatory aspects

Nano-farming nowadays is a recently developing topic that covers almost many aspects of agriculture that include such as food security, materials used for packaging, in treating diseases, as a smart delivery system, bioavailability, molecular and cellular biology techniques, and also for detection of pathogens *etc* [203]. The use of nano- products requires an urgent demand to better reveal the environmental effect on the biological system so as to device strategies for their safe implementation in the upliftment of agricultural and food sector. Some of the contemporary studies have revealed that NPs show toxic effects on the biological environment if used beyond their prescribed optimum concentration [204, 205]. Therefore, consumable nano- products available in the market having an unsure risk factor need the development of some regulatory frameworks. The existing regulatory frameworks on NPs related risk evaluation are primarily based on the process of their synthesis [206]. Human beings get easily exposed to NPs because the use, synthesis, degradation, and waste treatment of NPs are the major reasons for their release into the environment in different forms (**Fig. 2.4b**) [207]. Since the size of NPs is very minute, therefore they can be inhaled very smoothly and ultimately arrive in the bloodstream, liver, heart, or blood cells. The negative toxicity of NPs generally depends on their origin where many are nontoxic and others have a positive impact on health (**Fig. 2.4b**) [208]. With the constant manufacture and use of NPs during the recent decades, these are unavoidably released into the atmosphere. The possible toxicological effects of NPs on the atmosphere are very limited as they are least explored [209]. It may also be presumed that NPs may also destroy beneficial bacteria present in the environment which helps in various biological processes such as degradation of organic matter during waste treatment, nitrogen-fixing bacteria in soil, *etc* (**Fig. 2.4a**) [210]. However, developing species-specific NPs make them a potential tool to eradicate multidrug-resistant (MDR) microbes [211]. On the other hand, nanoparticles such as Al_2O_3, Ag, SiO_2, ZnO, TiO_2, etc. are known to have insecticidal activities [212] but the chances of the non-targeted killing off beneficial insects taking part in the pollination of plants cannot be ruled out [213]. Nanoparticles usually acquire higher chemical reactivity and bioactivity, high penetrating ability, and good bioavailability which make them superior for their wide use in medicine. On the other hand, these characteristics are also accounted for possible toxicity. Therefore, many major rules, laws, and regulatory frameworks have been imposed by numerous government organizations to reduce or restrict risks connected with NPs [214]. But, there are no exact worldwide laws and rules and no approved legal regulations for systematic production, proper handling or systematic labeling, toxicology testing, and assessment of the environmental effect of NPs. The biomedical field has modified its regulations relating to the application of NPs for actual consumable purposes [215, 216]. Presently, European Union (EU) and the USA have tough regulatory rules and principles of laws to manage the possible risks of NPs. The US regulatory government and agencies such as the Food and Drug Administration (FDA), the United States Environmental Protection Agency (USEPA), and the Institute for Food andAgricultural Standards (IFAS) have started developing laws and regulations to manage the toxicity and risks of NPs and its consumable products [214]. Evaluation of NPs toxicity and its side effects on the environment must be analyzed in the field of pharmacology, therapeutics, diagnostics, agriculture, cosmetics, etc. [217]. The U.S regulatory bodies like Environmental Protection Agency and the Food and DrugAdministration (FDA) and the European Commission regulatory bodies like Health & Consumer Protection Directorate have started correlating with the possible risks imposed by metal NPs [218]. However, WHO proposes a set of guidelines to policymakers and professionals in the field of occupational health and safety with recommendations on how to protect workers from the potential risks of metal-nanomaterials [88]. Scientists and producers are needed to be well-informed on the regulatory frameworks before the production and marketing of nano-food products. Therefore, evaluation of the probable risk is an urgent requirement to know the effect of NPs used in the future. Nanotechnology has not only improved the quality of foods by making them tastier, healthier, and more nutritious but has also helped a lot in generating new food products, and better packaging and storage techniques [219].

2.12 Potential risks on human health due to accumulation of NPs through food chain

There are only a handful of literature related to the interactions and communications of NPs in plant cell and also other organisms, therefore the consequence of metal NPs exposure in plants remain uncertain [220]. The majority of the literature reveals the seed germination upon exposure to NPs and only a handful of literature demonstrates the bioaccumulation and biotransformation on NPs into food crops [188]. The inadequate availability of these studies and data results in a limited concept on how NPs are translocated and bio-accumulated into various levels of the food chain [220]. Consequently, there is an immediate need to clarify how nano-sized particles can affect levels of the food chain and, eventually, assess human health risks.

2.13 Environmental fate of NPs

Depending on the large-scale use of NPs in any field, assessments of the qualitative and quantitative exposure of consumers to NPs and engineered NPs as well as an overall overview of their fate and their environmental impact are still marginal and unclear. Nanoparticles may directly enter into the environmental cycles during the disposal of nano-based products. Their fate depends on several factors such as type, size, coating material, the methods of their discharge, the discharge location, environmental conditions such as pH, ionic strength, interaction with organic matterof the disposed of area, bioaccumulation, and the extent of their trophic transfer, *etc* [220].

2.14 Migration characteristics of NPs

There are only a few studies available related to the characteristics of NPs migration in water ecosystems but the mechanisms may provide some precise view into this concept. The size of the NPs particle, its ionic strength, functional groups of NPs surface, and its organic matter are the various parameters that can influence the transportation of NPs into the environment. Larger NPs are found to have higher mobility due to their greater stability [221]. Nanoparticle transport gets inhibited with the increasing ionic strength [222]. The chances of migration of NPs used as a nano-fertilizer to underground water bodies however cannot be nullified [223].

2.15 Future scope and possibilities in nano-farming

Nanotechnology presents one of the exciting new fields of research that holds the potential to address many of the pressing needs in all areas of food and agriculture. In the food sector, nanotechnology has great potential to improve food functionality and quality. Its applications in agriculture include the use of nano-agrochemicals (nano-fertilizer, nano-pesticides, herbicides, *etc.*), nano-bio sensors, nano-formulated preservatives, and packaging materials, nutritional supplements, plant growth promoters, nano-enhanced bio-treatment for agricultural wastewater, etc. Nanotechnology appears as a technology that holds the potential to revolutionize the whole agri-food sector, improving the quality of food as the main goal [224].

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