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Research review paper

Perspectives for nano-biotechnology enabled protection and nutrition of plants

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ABSTRACT

Indiscriminate use of pesticides and fertilizers causes environmental pollution, emergence of agricultural pests and pathogens, and loss of biodiversity. Nanotechnology, by virtue of nanomaterial related properties, has potential agro-biotechnological applications for alleviation of these problems. The literature pertaining to the role of nanotechnology in plant and soil systems demonstrates that nanomaterials may assist in a) the controlled release of agrochemicals for nutrition and protection against pests and pathogens, b) delivery of genetic material, c) sensitive detection of plant disease and pollutants and d) protection and formation of soil structure. For instance, porous silica (15 nm) and biodegradable, polymeric chitosan (78 nm) nanoparticles displayed slow release of encapsulated pesticide and fertilizer, respectively. Further, nanosized gold (5–25 nm) delivered DNA to plant cells while iron oxide (30 nm) based nanosensors detected pesticides at minute levels. These functions assist the development of precision farming by minimizing pollution and maximizing the value of farming practice.

Contents

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1. Introduction

Conventionally, pathogens and pests are controlled by annual pesticide applications of ~2 million metric tons worldwide (worth US $ 35 billion), 90% of which are lost to the air during application and as run-off, affecting both the environment and application costs to the farmer (Stephenson, 2003). Indiscriminate pesticide usage increases pathogen and pest resistance, reduces soil biodiversity, diminishes nitrogen fixation; contributes to bioaccumulation of pesticides, pollinator decline and destroys habitat for birds (Tilman et al., 2002). Moreover, the application of excess volumes of fertilizers adds to the tribulations of the already delicate ecology as run-off (Tilman et al., 2002). The world demand for fertilizer was forecast to increase by 4.8% to 170.4 million metric tons by 2010/11 (Heffer and Prud'homme, 2010). Therefore there is an urgent need to tackle the excessive usage of pesticides and fertilizers by a) finding alternatives to current pesticide and fertilizer deployment, b) rapidly and locally detecting presence of pathogens and pests, as well as pesticides and nutrient levels; and c) developing methods for either agrochemical removal or degradation to promote soil health.

Biotechnological advancements in protection and nutrition strategies for plants have attempted to provide some solutions for these problems. Crop improvement for disease resistant or stress tolerant plants is one such approach. Transgenic insect resistant maize and cotton crops, with insecticidal genes from *Bacillus thuringiensis,* seek to replace insecticides with host-plant resistance that provides higher yields. However, transgenic crops are not accepted globally, yet. Other alternatives to agrochemicals are biopesticides and biofertilizers. Biopesticides, comprise living organisms or their derived products, are natural antagonists of pathogens and pests. Their key advantages include specificity, safety to mammals and other non-target organisms, environmental compatibility, applicability with chemical pesticides in integrated pest management and acceptance for organic agriculture. Similarly, biofertilizers comprise environment friendly microorganisms that supply or improve availability of nutrients to promote soil fertility and crop productivity. Biopesticides and biofertilizers are slowly gaining acceptance in terms of their applicability, efficiency and eco-friendly nature, though their on-field stability and shelf life are the major concerns. Other approaches for reducing agrochemical applications are development of monitoring systems for plant pathogens and agrochemicals that allow early intervention and optimum application. Also, biotechnology has sought to restore agro-chemically damaged soils with microorganisms or plants *i.e.* bioremediation or phytoremediation, respectively. Although these biotechnological advances are evident, the present picture that remains is that of a rapidly degrading and polluted ecosystem caused by prevailing practices. To tackle the situation we need to harness innovative approaches towards agriculture such as nanotechnology.

Nanotechnology, the process to generate, manipulate, and deploy nanomaterials, represents an area holding significant promise for the agricultural scenario (Table 1, Baruah and Dutta, 2009; Navrotsky, 2000; Kuzma, 2007). Nanotechnology employs nanoparticles (NPs) having one or more dimensions in the order of 100 nm or less (Auffan et al., 2009). Other authors refer to NPs as colloidal particulate systems with size ranging between 10 and 1000 nm (Nakache et al., 1999). Nanomaterials hold great promise regarding their application in plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties. A wide variety of materials are used to make NPs, such as metal oxides, ceramics, silicates, magnetic materials, semiconductor quantum dots (QDs),lipids, polymers, dendrimers and emulsions (Niemeyer and Doz, 2001; Oskam, 2006; Puoci et al., 2008). Polymers display controlled release of ingredients, a character useful for developing polymeric NPs as agrochemical carriers. Metal nanoparticles display size dependent properties such as magnetism (magnetic NPs), fluorescence (QDs) or photocatalytic degradation (metal oxide NPs) that have biotechnological applications in sensor development, agrochemical degradation and soil remediation (Table 1).

Table 1
Applications of nanotechnology in agriculture.

Application	Nanoparticles	Reference
Pesticide delivery		
Chemical		
Avermectin	Porous hollow silica (15 nm)	Li et al. 2007
Ethiprole or phenylpyrazole	Poly-caprolactone (135 nm)	Boehm et al., 2003
Gamma cyhalothrin	Solid lipid (300 nm)	Frederiksen et al. 2003
Tebucanazole/chlorothalonil	Polyvinylpyridine and polyvinylpyridine-co-styrene (100 nm)	Liu et al. 2001
Biopesticides		
Plant origin: nanosilica for insect control *Artemisia arborescens*	Nanosilica (3–5 nm)	Barik et al., 2008
essential oil encapsulation	Solid lipid (200–294 nm)	Lai et al. 2006
Microganisms: *Lagenidium giganteum* cells in emulsion	Silica (7–14 nm)	Vandergheynst et al., 2007
Microbial product: absorption of *Myrothrecium verrucaria* enzyme complex	Chitosan/kaolin (250–350 nm)	Ghormade et al.unpublished
Fertilizer delivery		
NPK controlled delivery	Nano-coating of sulfur (100 nm layer)	Wilson et al. 2008
	Chitosan (78 nm)	Corradini et al. 2010
Genetic material delivery		
DNA	Gold (10–15 nm)	Torney et al. 2007
	Gold (5–25 nm)	Vijayakumar et al. 2010
	Starch (50–100 nm)	Liu et al. 2008
Double stranded RNA	Chitosan (100–200 nm)	Zhang et al. 2010
Pesticide sensor		
Carbofuran/triazophos	Gold (40 nm)	Guo et al. 2009
DDT	Gold (30 nm)	Lisa et al. 2009
Dimethoate	Iron oxide (30 nm), zirconium oxide (31.5 nm)	Gan et al. 2010
Organophosphate	Zirconium oxide (50 nm)	Wang et al. 2009
Paraoxon	Silica (100–500 nm)	Ramanathan et al. 2009
	Carbon nanotubes	Joshi et al. 2005
Pyrethroid	Iron oxide (22 nm)	Kaushik et al., 2009
Pesticide degradation		
Lindane	Iron sulfide (200 nm)	Paknikar et al. 2005
Imidacloprid	Titanium oxide (30 nm)	Guan et al. 2008

Potential applications of nanotechnology in agriculture are: delivery of nanocides–pesticides encapsulated in nanomaterials for controlled release; stabilization of biopesticides with nanomaterials; slow release of nanomaterial assisted fertilizers, biofertilizers and micronutrients for efficient use; and field applications of agrochemicals, nanomaterials assisted delivery of genetic material for crop improvement (Fig. 1). Nanosensors for plant pathogen and pesticide detection, and NPs for soil conservation or remediation are other areas in agriculture that can benefit from nanotechnology (Fig. 1). Enzyme immobilization for nanobiosensor using nanomaterials involves the high value low volume application of enzymes (Kim et al., 2006). Usually costly, large enzyme volumes are required for biocontrol in agricultural fields that would be practical if spray applications combined high volume with low value. Cost-effectiveness of such biocontrol preparations can be achieved by immobilization of enzyme/inhibitors on nanostructures, providing large surface areas, to increase the effective concentration of the preparation. In this review, we focus on nanomaterial-based technologies and their existing and potential applications in plant protection and nutrition.

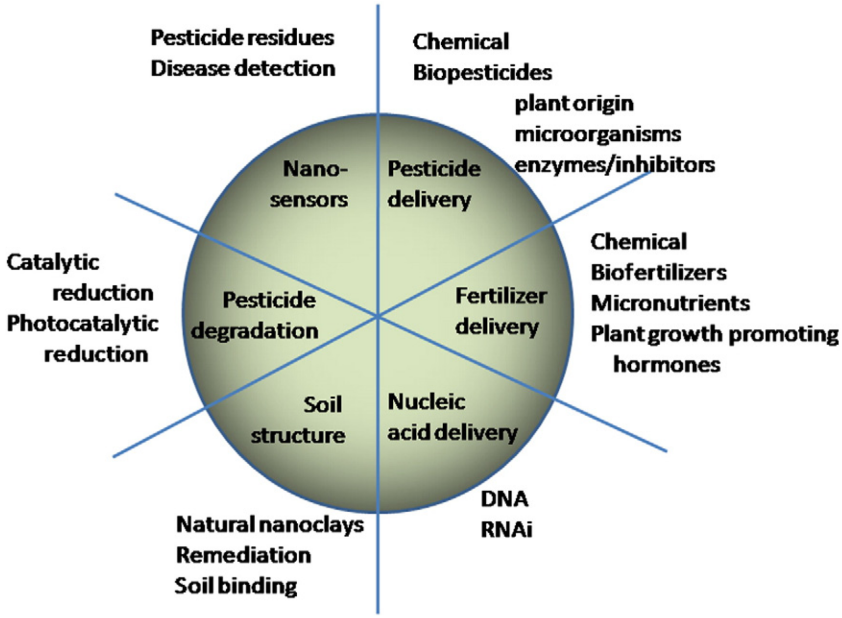

Fig. 1. Applications of nano-biotechnology in plant protection and nutrition.

2. Nanomaterials–nanoparticle preparation and properties

Nanomaterials of inorganic and organic origin are used for NP synthesis by a variety of physical and chemical methods (Table 2). Synthesis of NPs involve size reduction by top-down methods such as milling, high pressure homogenization and sonication while bottom-up processes involve reactive precipitation and solvent displacement (Sasson et al., 2007). Among inorganic materials, metal oxide NPs such as, TiO_2, ZnO, AgO and MgO and others are of particular interest as they are physically and optically stable with tunable optical properties (Makhluf et al., 2005; Stoimenov et al., 2002). Photocatalytic (TiO_2, ZnO) and microbiocidal (AgO and MgO) NPs are employed for pesticide degradation, detection and control food spoilage, respectively (Baruah and Dutta, 2009; Makhluf et al., 2005). Among metal oxide NPs, the effects of fine-tuning the material's size, to obtain desirable properties, are advantageous in

Table 2
Nanomaterials, methods of nanoparticles preparation and their applications.

Nanomaterial	Synthesis method	Remarks	Ref
Inorganic			
Metal nanoparticles AgO, TiO_2, ZnO, CeO_2; Fe_2O_3 FePd, Fe–Ni (magnetic); Silica; CdTe, CdSe (QDs)	Physical: Arc-discharge, high energy ball milling, laser pyrolysis/abalation. Chemical: electrochemical, chemical vapor deposition sonochemisty, microemulsion sol–gel, reverse precipitation	Photothermal therapy, Imaging studies, delivery of biomolecules (proteins, peptides nucleic acids), biosensors,diagnostic procedures, implants, pesticide degradation	Niemeyer and Doz, 2001 Oskam, 2006 Velasco-Garcia and Mottram, 2003 Veerapandian and Yun, 2009
Clay			
Montmorillonite layered double hyroxides	Physical: exfoliation co-precipitation	Delivery of pesticides, fertilizers, plant growth promoting factors	El-Nahhal et al., 1999 Lakraimi et al., 2000
Organic			
Carbon nanotubes, nanofibres	Arc-discharge, laser abalation chemical vapor deposition	Biocatalysts, sensing, neural/orthopedic implants atomic force microscope probes	Loiseau A et al., 2006
Lipid			
Liposomes Lipopolyplexes Solid lipid nano-particles	Chemical: sonochemisty, reverse phase evaporation high pressure homogenization	Delivery of DNA, xenobiotics, and pesticides, essential oils, transfection	Yu et al. 2009 Lai et al., 2006
Polymeric			
Natural			
Cellulose,Starch Gelatin, Albumin Chitin, chitosan	Chemical: suspension, emulsion, dispersion -precipitation	Biocompatible, biodegradable non-toxic for drug delivery delivery of DNA/RNA	Liu et al. 2008 Puoci et al., 2008
Synthetic			
Dendrimers (PAMAM,PPI) Polyethylene oxide Polyethylene glycol Polylactides Polyalklycyanoacrylates		Delivery of therapeutic/ diagnostic agents, pesticides delivery of DNA/RNA	

QDs-semiconductor quantum dots, PAMAM—polyamidoamine, PPI—polypropylene imine.

biotechnology for sensor development, agrochemical degradation and soil remediation (Table 2). The high stability, chemical versatility and biocompatibility of silica NPs are employed in pesticide delivery (Li et al., 2007). Inorganic, semiconductor QDs that are brighter, more stable against photobleaching, and one-third as wide in spectral width, when compared to conventional fluorophores, are making a significant impact in bioanalytical research and development (Vinayaka et al., 2009). Ultrasensitive QD bioconjugates, when labeled with immunomolecules that recognize specific antibodies or antigens, bear potential for development as sensors for disease detection or agrochemicals (Vinayaka et al., 2009).

Other inorganic materials such as montmorillonite and other clay nanoparticles have a structure of stacked platelets with one dimension of the platelet in the nanometer scale. Nano-clays have a high aspect ratio that provides more interactive surfaces when exfoliated and dispersed well. Loading of chemicals between layers of both materials is in a pH dependent manner. El-Nahhal et al. (1999) demonstrated that nano-clay formulations reduced the dispersion of the pollutant in the environment and removed the toxic compound (Table 2). Layered double hydroxides are anionic clays made of stacks of octahedral sheets in nanometer scale produced by co-precipitation of the component elements (Choy et al., 2007). Several studies demonstrated their potential use in the deployment of agrochemicals such as fertilizers, plant growth promoters, and pesticides (Bin Hussein et al., 2009; Lakraimi et al., 2000; Olanrewaju et al., 2000).

Organic materials such as carbon nanotubes, lipids and polymers are versatile materials with multiple applications (Table 1). The unique electronic, metallic and structural characteristics make carbon nanotubes an important class of materials (Hatton et al., 2008). The possibility of electron transfer reactions due to their structure dependent metallic character and their high surface area provides ground for unique biochemical sensing systems (Joshi et al., 2005).

Solid lipid NPs are delivery systems that comprise of aqueous dispersions of solid lipids or dry powders, such as triglycerides, steroids, waxes, long chain fatty acids, and emulsifiers prepared by high pressure homogenization. Solid lipid NPs were reported as delivery systems for pesticide and biocidal essential oils (Table 2, Frederiksen et al., 2003; Lai et al., 2006).

Polymeric NPs made from natural and synthetic polymers by wet synthetic routes are widely used due to their stability and ease of surface modification (Table 2, Herrero-Vanrell et al., 2005; Vauthier et al., 2003). NPs prepared from biopolymers or natural sources possess advantages, such as availability from replenishable agricultural (cellulose, starch, pectin) or marine (chitin and chitosan) resources, biocompatibility, biodegradability and ecological safety. Biodegradable polymers are studied mainly for fabrication of delivery systems for controlled release of active ingredients, stabilization of labile molecules (*e.g.*, proteins, peptides, or genetic material). Depending on the method of preparation nanospheres (round NPs), nanofibres (~100 nm long fibers) or nanocapsules (hollow NPs) can be constructed to possess different properties and release characteristics for the best delivery or encapsulation of the active ingredient.

3. Nanoparticles based delivery systems: applications and advantages

Delivery systems in agriculture are important for application of pesticides and fertilizers as well as during genetic material mediated plant improvement. Application systems for pesticides need to focus on efficacy enhancement and spray drift management while fertilizers face problems of bioavailability due to soil chelation, over-application and run-offs. A viable alternative for these problems is provided by controlled delivery systems for the pesticide and fertilizer application. Controlled delivery technique aims towards measured release of necessary and sufficient amounts of agrochemicals over a period of time, to obtain the fullest biological efficacy and to minimize the harmful effects (Tsuji, 2001). For this purpose micronic and submicronic particles were explored as agrochemical delivery vehicles. In comparison to micronic particles (≥1000 nm), nanoparticles (<1000 nm) offered the advantage of effective loading due to the larger surface area, easy attachment and fast mass transfer. As depicted in Fig. 2, the active ingredient is adsorbed, attached, encapsulated or entrapped unto or into the nano-matrix (Fig. 2). Controlled release of the active ingredient is achieved due to the slow release characteristics of the nanomaterial, bonding of the ingredients to the material and the environmental conditions.

In case of genetic material, delivery systems face challenges such as limited host range, transportation across cell membrane and trafficking to the nucleus. Nevertheless, the use of NPs assisted delivery for genetic material to develop insect resistant plant varieties is being studied. For instance, DNA-coated gold NPs are used as bullets in 'gene gun' system, for bombardment of plant cells and tissues to achieve gene transfer (Vijayakumar et al., 2010).

3.1. Delivery of pesticides/biopesticides

Currently, there are increasing reports for resistance development to the prevalent groups of insecticides and fungicides that are applied for pest and pathogen control (Smith et al., 2008). In addition, the stringency of regulatory bodies like Central Insecticide Board (CIB, India), Food and Drug Administration (FDA, USA) though rightly, has increased for the registration of pesticides (Finney, 1994; Racke, 2003). Therefore there is an urgent need to impart a benign safety profile and improved biological efficacy to the existing chemical and biological pesticides with possible use of delivery systems.

Nanotechnology has potential for efficient delivery of chemical and biological pesticides using nanosized preparations or nanomaterial based agrochemical formulations (Table 1). The benefits of nanomaterial based formulations are the improvement of efficacy due to higher surface area, higher solubility, induction of systemic activity due to smaller particle size

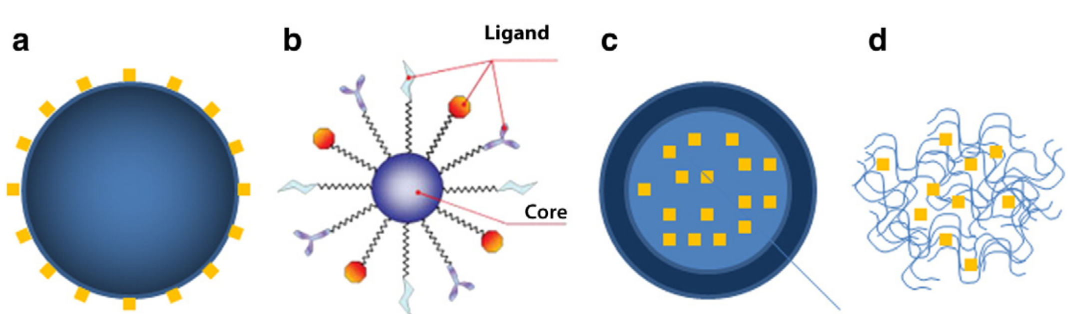

Fig. 2. Schematic representation of different nanodevices for delivery of pesticides, fertilizers or nucleic acids (a) adsorption on nanoparticle; (b) attachment on nanoparticle mediated by different ligands; (c) encapsulation in nanoparticulate polymeric shell; (d) entrapment in polymeric nanoparticle.

and higher mobility and lower toxicity due to elimination of organic solvents in comparison to conventionally used pesticides and their formulations (Sasson et al., 2007). In case of biopesticides, NPs can play a major role in enhancing the efficacy and stability of whole cells, enzyme and other natural products used. However in the field, application of NPs for the delivery of pesticides and biopesticides face several challenges such as multiple environmental perturbations, large areas under spray coverage and finally cost effectiveness. In the usual spraying regime the whole crop is sprayed with the chemical for the ease of application involving a high volume, low value preparation. Whereas nanomaterial based preparations are expected to involve a low volume, high value applications. Such controlled nanoparticulate delivery systems will require a targeted delivery approach focused using the knowledge of the life-cycle and the behavior of the pathogen or pest.

3.1.1. Chemical pesticides

Characteristics of a successful modern crop protection chemical are: to remain active in the spray environment (sun, heat, rain), penetrate the organism (fungus, insect), transport to the target, resist defense of the pest/pathogen, remain benign to plants and mammals, be cost effective to formulate and manufacture, preferably possess a new mode of action, and provide economic returns and social benefits (Smith et al., 2008). These requirements prompted the development of the concept of 'Pesticide Delivery System' (PDS) (Tsuji, 2001). PDS makes the active ingredients available to a specified target at concentrations and durations designed to accomplish the intended effect by maintenance of the fullest biological efficacy and reduction of various harmful effects. PDSs face the main difficulty of application to an open environmental system (Tsuji, 2001). Initially, the controlled release formulations with microcapsules (>10 μm) encapsulating pesticides were developed for delivery (Gimeno, 1996; Tefft and Friend, 1993; Tsuji, 2001). Fessi et al.(1989) developed a new nanoprecipitation method, that involved formation of spontaneous nano-emulsions with polycondensation at oil–water interface, to prepare NPs (200–300 nm) in an easy and reproducible way. The insecticide ethiprole, a phenylpyrazole compound that blocks the insect γ — aminobutyric acid receptor and neurotransmission, faced problems of photoinactivation during field applications (Caboni et al., 2003). The improved delivery of ethiprole to plants was assessed using nanospheres for protection against photoinactivation (Boehm et al., 2000; 2003). Stable polymeric poly-caprolactone and polylactic acid nanospheres (135 nm), encapsulating 3.5% of ethiprole, were obtained with the nanoprecipitation method. Initial biological testing for aphid control on cotton plants indicated that speed of action and controlled release of nanosphere formulations were not at par with the chemical application (Table 1). Nevertheless, the nanosphere formulation showed enhanced systemicity of the active ingredients and improved its penetration through the plant, due to their small size (Boehm et al., 2003).

Pesticides with a short half life such as avermectin (6 h), the insect chloride channel inhibitor that blocks neurotransmission, faced problems of UV inactivation on the fields. Porous hollow silica NPs were reported to protect avermectin from UV degradation and allowed its slow release (Table 1). Porous hollow silica NPs with a shell thickness of ~15 nm and a pore diameter of 4–5 nm had an encapsulation capacity of 625 g kg^{-1} for avermectin. Slow release of encapsulated avermectin by the NPs carrier was reported for about 30 days (Li et al., 2007).

NPs based on polymeric materials polyvinylpyridine and polyvinyl-pyridine-co-styrene (100–120 nm) displayed controlled release of the fungicides tebuconazole and chlorothalonil for solid wood preservation (Table 1, Liu et al., 2001). Recently, polymeric nanocapsules were suggested as vehicles for the pesticides ivermectin and acetamiprid (Zheng and Shang, 2005). While Wang et al. (2004) suggested nanosized inorganic particles such as TiO_2, SiO_2, Fe_2O3, or Al_2O_3 as pesticide carriers for increased bioactivity and reduction in residues.

Further, solid lipid NPs (300 nm) prepared from glyceryl behenate were used by Frederiksen et al. (2003) to encapsulate γ — cyhalothrin with limited success due to partial expulsion of the active ingredient (Table 1). However, this formulation exhibited >10 times reduced toxicity towards fish and daphnia, as compared to the standard formulation, without an apparent loss in insecticidal activity. The use of liposomal formulations as delivery systems for pesticides had limited applications due to their high cost (Pons and Estelrich, 1996).

Other materials such as nano-clays and layered double hydroxides possess good biocompatibility, low toxicity and potential for controlled release. (Bin Hussein et al., 2002; Choy et al., 2007). Controlled release for nano-clays was engineered by surface coating with different polymers that manipulated electrostatic interactions between the chemical load and clay particles (Lee and Fu, 2003). In addition, nano-clays protected the agrochemicals against UV-degradation (El-Nahhal et al., 1999). Layered double hydroxides with high affinity for anionic species are soluble in acidic conditions. In the case of hydrophobic chemicals, the arrangement prevented re-crystallization, increased solubility and therefore bioavailability. Agrochemicals such as fertilizers (nitrate), plant growth promoters (α-naptheleneacetate), and pesticides (4-(2,4-dichlorophenoxy) butyrate) were deployed using double layered hydroxides (Bin Hussein et al., 2002, 2009;Lakraimi et al., 2000; Olanrewaju et al., 2000).

In addition to the use of NPs for pesticide delivery, the use of nanosized aqueous dispersion formulations was suggested to enhance the bioavailability of pesticides (Storm et al., 2001). Organic solvents such as toluene or xylene are used for pesticide preparations (Kent and Reigel, 2003). Nanosized aqueous dispersion or nanosuspensions eliminated the need for organic solvents and provided a process for stabilizing formulations of two or more immiscible pesticides. Storm et al. (2001) used milling technologies in presence of grinding media (polymer beads) and surface active agents to obtain stable suspensions of various fungicides and insecticides with particle sizes around 148–314 nm. The efficacy of the spinosad insecticide, causing excitation of insect nervous system, was found to be dependent on particle size. When particle size of the spinosad nanosuspension applied on spider mites, was 404 nm the LC_{50} was 15 mg l^{-1} that further decreased to 11 mg l^{-1} for 372 nm, 7.6 mg l^{-1} for 332 nm and 4 mg l^{-1} for 163 nm (Storm et al., 2001).

Thus, the use of nanosuspensions, nanoparticles and polymeric nanoparticles has potential for developing safer chemical pesticide formulations for application in the field. Recently, one of the pesticide companies released a nano-sized aqueous dispersion formulation with broad spectrum systemic fungicidal action (Banner MAXX from Syngenta), for the control of leaf spots, blights, rusts and powdery mildew diseases on ornamentals, turf, and other landscape plantings (Latin, 2006; Wong and Midland, 2004, 2007).

3.1.2. Biological

In the rapidly developing field of nanobiotechnology the integration of biomolecules such as enzymes, metabolites *etc.* or whole cells with nanostructures leads to hybrid systems that have numerous applications in many fields including agriculture (Bailey et al., 2010).

3.1.2.1. Plant origin. Plants provide a non-toxic source of molecules with proven biological efficacy and that are usually non-persistent in fresh water and soil (Isman, 2000). However, phytochemicals such as secondary metabolites and essential oils face problems of stability and cost effectiveness. In case of essential oils, their chemical instability in the presence of air, light, moisture, and high temperatures that causes rapid evaporation and degradation of some active components is a major concern. Incorporation of essential oils into a controlled-release nano-formulation prevents rapid evaporation and degradation; enhances stability and maintains the minimum effective dosage/application. Solid lipid NPs (200 nm), composed of long chain fatty acids and emulsifiers, were used to protect labile compounds such as tocopherol acetate, retinoids, and vitamin E from degradation (Jenning and Gohla, 2001;

Wissing and Müller, 2001). An essential oil from *Artemisia arborescens*, faced the problem of instability during pesticidal acitivty against *Aphis gossipy* (citrus fruit pest), adult and young *Bemisia tabaci*, and *Lymantria dispar* (cork plant pest) (Lai et al., 2006). Incorporation of *A. arborescens* essential oil into solid lipid NPs (200–294 nm) reduced the rapid evaporation of essential oil, in comparison to the reference emulsions (Table 1, Lai et al., 2006).

The essential oil from garlic was loaded on polymer NPs (240 nm) coated with polyethylene glycol (PEG) to evaluate their insecticidal activity against adult *Tribolium castaneum* (Yang et al., 2009). The oil-loading efficiency reached 80% at the optimal ratio of essential oil to PEG (10%). The control efficacy of garlic essential oil loaded NPs against adult *T. castaneum* remained over 80% after five months, due to the controlled slow release of the active components, in comparison to free garlic essential oil (11%).This indicated the feasibility of PEG coated NPs loaded with garlic essential oil for control of storage pests.

Amorphous nanosilica is obtained from various natural sources like the shell wall of phytoplankton, epidermis of vegetables, burnt pretreated rice hulls and straw at thermoelectric plants and volcanic soil. Amorphous nanosilica displayed promising potential as a biopesticide (Barik et al., 2008). The silica NPs were physio-sorbed by the cuticular lipids disrupting the protective barrier and thereby causing death of insects purely by physical means (Barik et al., 2008). Application of NPs on the leaf and stem surface did not alter either photosynthesis or respiration in several groups of horticultural and crop plants. They did not cause alteration of gene expression in insect trachea and were, thus, qualified for approval as the nanobiopesticide. Use of amorphous silica as a nanobiopesticide is considered safe for humans by World Health Organization (WHO). Debnath et al. (2011) reported that silica NPs caused 100% mortality in rice weevil, *Sitophilus oryzae*. Furthermore, surface charged modified hydrophobic silica NPs (3–5 nm) were successfully used to control a range of agricultural insect pests and animal ecto-parasites of veterinary importance (Ulrichs et al., 2006). It was successfully applied as a thin film on seeds to decrease fungal growth and boost cereal germination (Robinson, 2010). Nano-silica may be useful against stored grain, household pests, animal parasites, fungal organisms, worms, *etc*.

Nuclear polyhedrosis virus (BmNPV) infects silkworms, *Bombax mori* and causes heavy losses to the silk industry. BmNPV infection enhances the level of certain lipids in silkworms. Hui-Peng et al. (2006) showed that lipase treatment, a viable option for controlling BmNPV, interfered in the hormonal balance and could not be applied to pre-molting stage. Plant-stem-derived nanosilica was capable of reducing certain classes of lipoproteins (in native form) present in the silkworm larval hemolymph (Barik et al., 2008). Nanoparticles circulate easily in lepidopteran system and would be less harmful in the hemocoel (Ulrichs et al., 2006). When applied to BmNPV afflicted silkworms nanosilica (40–60 nm) increased the survival from 0 to 30% (Goswami et al., 2010).The silkworm model might be used successfully for controlling other insect, animal and human diseases where an increase in host lipids was utilized for pathogen growth. Nano-silica has potential to control malaria that causes alterations in host lipid profiles (Barik et al., 2008).

3.1.2.2. Microbes and microbial products as biocontrol agents. Bacteria, viruses and fungi can function as biological control agents against insect pests. Bacterial and viral formulations need to be ingested by the host and are susceptible to dessication, heat, and UV inactivation. The use of nanoformulations may offer new ways to enhance the stability of these biological agents. Fungal biocontrol agents or mycopesticides are promising as they act by contact and do not need ingestion, can be easily mass produced, and are relatively specific (Deshpande, 1999, 2005; Nahar et al., 2004). Insect infecting or entomopathogenic fungal genera such as *Metarhizium*, *Beauvaria*, *Nomuraea, Paecilomyces* and *Verticillium* spread infection among the insect host population by means of conidia that require the presence of moisture for their germination to initiate host pathogenesis (Kulkarni, 2008). Parameters including time of application and formulation are utilized to maintain the moisture levels for promotion of germination of the infective propagules (Deshpande, 2005). The application of NPs to address these issues holds promise for further development of eco-friendly formulations.

Lagenidium giganteum (Oömycetes: Lagenidiales), a mosquito biolarvacide registered with the US Environmental Protection Agency (USEPA registration No. 56984–2) had limited commercial use due to its poor stability during storage and expensive storage requirements (Vandergheynst et al., 2007). *L. giganteum*, a water mold, was poorly tolerant to desiccation. Addition of hydrophobic silica NPs (7–14 nm) to the water in oil emulsion formulation of *L. giganteum* mycelium reduced the desiccation. These NPs thickened the formulation, reduced cell sedimentation and imparted >95% efficacy at infecting mosquitoes after 12 weeks of storage at room temperature (Vandergheynst et al., 2007).

Microbial products such as enzymes, inhibitors, antibiotics and toxins are promising as biopesticides against plant pests and pathogens. The Actinomycete *Streptomyces* is known to secrete miticidal antibiotic (tetranactin) and broad spectrum insecticides (avermectin, milbemycin) (Devakumar and Parmar, 1993). The insecticidal properties of bacterial toxin (Bt) are well known. The specificity of fungal interactions *viz.* mycoparasitism of *Trichoderma* with plant pathogenic fungi or entomopathogenesis of *Metarhizium* to insect pests are sources of effective biocontrol agents such as mycolytic and cuticle-degrading enzymes (ME, CDE) antibiotics (viridian, gliovirin) and toxins (destruxins, bassianin) (Amiri-Besheli et al., 2000; Benitez et al., 2004; Deshpande, 1998a, 1999; Howell, 2006; Kulkarni et al., 2008; Nahar et al., 2004; Patil et al., 2000; Vey et al., 2001). For biocontrol purposes, microbial products need stabilization and directed delivery mechanism towards identified targets. *Myrothecium verrucaria,* a saprophytic fungus, produced endochitinase that killed *Aedes aegypti*, mosquito larvae within 48 h (Chavan, 2009; Deshpande, 1998b; Mendonsa et al., 1996). A study is being conducted to explore the potential of biocompatible and biodegradable nanomaterials like chitosan or clay as enzyme stabilizing and delivery agents. Chitosan (250–350 nm) and montmorillonite clay NPs (100–200 nm) were used to prepare nanoformulations that stabilized the *Myrothecium* enzyme complex (Table 1). In the bioassay, biological activity against the plant pathogen *Fusarium* and cotton mealy bug, *Phenacoccus gossypiphilous* was observed due to controlled slow release of the enzymes (Ghormade et al., unpublished data).

3.2. Delivery of fertilizers

Localized application of large amounts of fertilizer, in the form of ammonium salts, urea, and nitrate or phosphate compounds are harmful. Much of the fertilizers are unavailable to plants as they are lost as run-off causing pollution (Wilson et al., 2008). Nanomaterials have potential contributions in slow release of fertilizers. Nanocoatings, or surface coatings of nanomaterials, on fertilizer particles hold the material more strongly from the plant due to higher surface tension than conventional surfaces. Moreover, nanocoatings provide surface protection for larger particles (Brady and Weil, 1999; Santoso et al., 1995).

3.2.1. Chemical

Fertilizers with sulfur nanocoating (≤100 nm layer) are useful slow release fertilizers as the sulfur contents are beneficial especially for sulfur deficient soils (Table 1, Brady and Weil, 1999; Santoso et al., 1995). The stability of the coating reduced the rate of dissolution of the fertilizer and allowed slow sustained release of sulfur coated fertilizer. In addition to sulfur nanocoatings or encapsulation of urea and phosphate and their release will be beneficial to meet the soil and crop demands. Other nanomaterials with potential application include kaolin and polymeric biocompatible NPs (Wilson et al., 2008). Corradini et al. (2010) used biodegradable, polymeric chitosan NPs (~78 nm) for controlled release of the NPK fertilizer sources such as urea, calcium phosphate and potassium chloride (Table 1).

3.2.2. Biological

Biofertilizers are live formulations of beneficial microorganisms such as the fungal mycorrhizae, plant growth promoting rhizobacteria *Rhizobium, Azotobacter, Azospirillum* and blue green algae, phosphate solubilizing bacteria *Pseudomonas* sp and *Bacillus* sp (Wu et al., 2005). Microorganisms safely convert organic matter into simple compounds that provide plant nutrition, improve soil fertility, maintain the natural habitat of the soil and increase crop yield. The formulation of biofertilizer inocula, their storage and method of application are critical to the success of the product (Jha and Prasad, 2006). Some constraints in their widespread usage are short shelf life, lack of suitable carrier materials, susceptibility to high temperature, problems in transportation and storage. Also desiccation is another problem that occurs during storage. Potential application of polymeric NPs are for coating of biofertilizer preparations to yield formulations that are resistant to desiccation.

Micronutrients like manganese, boron, copper, iron, chlorine, molybdenum, and zinc promote optimum plant growth. Steady increase of crop yields following the green revolution has progressively depleted the soil of micronutrients like zinc, iron and molybdenum (Alloway, 2008). Farming practices, such as liming acid soils, contribute to micronutrient deficiencies in crops by decreasing the availability (Alloway, 2008). Foliar application of micronutrients, now a common horticultural practice, enhanced its uptake by the leaves (Martens and Westermann, 1991). Furthermore, the nanoformulations of micronutrients may be used as crop sprays for enhanced foliar uptake (Peteu et al., 2010). Supplementation of soils with micronutrients trapped in nanomaterials for their slow release would promote plant growth and soil health (Peteu et al., 2010).

3.3. Field application of pesticides and fertilizers

The mode of pesticide and fertilizer application influences their efficiency and environmental impact (Ihsan et al., 2007; Matthews, 2008; Matthews and Thomas, 2000). Currently spraying of pesticides involves either knapsacks that deliver large droplets (9–266 μm) associated with splash loss or ultralight volume sprayers for controlled droplet application with smaller droplets (3–28 μm) causing spray drift (Hoffmann et al., 2007). Constraints due to droplet size may be overcome by using NP encapsulated or nanosized pesticides that will contribute to efficient spraying and reduction of spray drift and splash losses (Fig. 3). The synthetic pyrethroid bifenthrin, used to protect cotton crops since 1980s, displayed low water solubility. The oil in water emulsion spray application on foliage was inadequate due to the poorly controlled drop size distribution and also posed the risk of transdermal exposure. Bifenthrin NPs suspension formulations for spraying formed stable dispersions and displayed increased efficiency due to interaction of active ingredients with insects and enhanced release of highly hydrophobic compounds (Liu et al., 2008). Further, NP suspensions were suggested to diminish the applicators' chemical exposure, since the particles were too large to penetrate through the skin, relative to compounds solubilized in organic solvents.

Another practical problem faced during pesticide application in the field is settlement of formulation components in the spray tank and clogging of spray nozzles. The recent nano-sized fungicide (~100 nm, BannerMAXX, Syngenta) prevented spray tank filters from clogging, did not required mixing and did not settle out in the spray tank due to the smaller sized particles (Robinson and Salejova-Zadrazilova, 2010). Furthermore, this fungicide did not separate from water for up to one year due to nano-size, whereas fungicides that contained larger particle size ingredients typically required agitation every 2 h to prevent clogging in the tank.

Proper application method of optimum quantities of fertilizer maximized nutrient uptake and reduced pollution (Ihsan et al., 2007). The choice of fertilizer application method mainly depends on: soil, crop, irrigation type and the nutrient applied. Current practices involving broadcasting, banding, side-dressing and dusting face problems of run-off due to dissolution in soil moisture and leaching. Placement of large amounts of fertilizer near the seeds and reduction in soil moisture caused salt damage. The effect of different methods of nitrogen fertilizer application on the algal flora and biological nitrogen fixation in wetland rice soil was studied in pot and field experiments. In the broadcast method of urea application, nitrogen fixation was inhibited while growth of green algae was favored. In contrast, deep placement of urea granules (1–2 g) did not suppress the growth of nitrogen fixing blue green algae (Roger et al., 1980). Placement NP coated fertilizers may contribute to slow release of the fertilizer preventing the rapid dissolution and therefore harm to the environment (Corradini et al., 2010; Wilson et al., 2008).

3.4. Delivery of genetic material

Delivery of genetic material such as DNA and small interfering RNA is important for the development of pest, pathogen and stress resistant strains of crop plants by alteration of gene expression (Gelvin, 2003; Price and Gatehouse, 2008). Gene delivery systems for plant transformation face obstacles such as targeting of delivery system, transportation through the cell membrane, uptake and degradation in endolysosomes and intracellular trafficking of DNA to the nucleus. Viral gene delivery vectors have a narrow host range, allow limited size of inserted genetic material and face the possibility of resurgence of

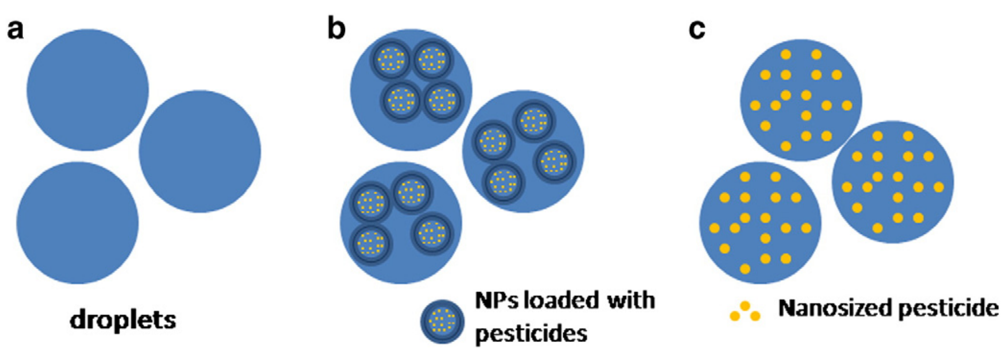

Fig. 3. Schematic representation of incorporation of nanoformulations in spray droplets to maximize efficient spraying. (a) spray droplets; (b) droplets containing nanoparticles loaded with pesticide; (c) droplets containing nanosized pesticides.

viral symptoms. Other methods applied for plant genetic transformation include: microinjection, *Agrobacterium* mediated transformation and microprojectile bombardment (Christou et al., 1988; Gelvin, 2003). These methods had either very low efficiency (0.01%–20% efficiency) or were applied only for dicotyledonous plant transformation (Sivamani et al., 2009).

To circumvent the above mentioned obstacles, NPs were employed to develop efficient gene transformation vehicles. Use of NPs expands the application of this technology to both dicotyledonous and monocotyledonous plants and also makes it tissue specific. Gold NPs (5–25 nm) embedded carbon matrices were employed for the delivery of DNA during transformation of plant cells that carried higher amount of genetic material as compared to the microparticles (Table 1, Vijayakumar et al., 2010). The DNA coated NPs gained easy access to the plant cell due to its size increasing the transformation efficiency in both monocotyledonous *Oryza sativa* (rice), and dicotyledenous *Nicotiana tabacum* (tobacco) and *Leucaena leucocephala* (white lead tree). NPs had lower plasmid and gold requirement as compared to the commercial micrometer-sized gold particles (Vijayakumar et al., 2010). Moreover, plant-cell damage with the NPs was minimal with increased plant regeneration. Further, Torney et al. (2007) demonstrated that a honeycomb surface-functionalized mesoporous silica NP system with 3 nm pores capped with disulphide bond held gold NPs (10–15 nm) transported DNA and chemicals into isolated plant cells and intact leaves with the help of gene gun (Table 1). Uncapping of the gold NPs, by disulphide reduction in cellular environment, released the chemicals and triggered gene expression in the plants under controlled release conditions (Torney et al., 2007). Liu et al. (2008) reported the that starch NPs (50–100 nm) conjugated with fluorescent material Tris-(2,2′-bipyridine) ruthenium — (Ru $(bpy)_3)^{2+}$ was used to transport plasmid DNA through *Dioscrea* sp plant cell wall, cell membrane and nuclear membrane in presence of ultrasound (Table 1). Apart from the advantage of acceptance by a wide host range the starch $(Ru(bpy)_3)^{2+}$ NPs also offered the possibility of tracking in the cells.

In addition to these synthetic vectors, nanoparticulate cationic liposomes and cationic polymers are also pursued as alternatives. Their permanent cationic charges interact electrostatically with negatively charged DNA to form complexes (lipo- or polyplexes). Although liposomes formed from cationic phospholipids offer advantages such as ease of preparation, their toxicity and relative low transfection efficiencies are disadvantageous as gene delivery vectors (Jong et al., 2007). Polymer/DNA complexes are more stable than those involving cationic lipids, and they protect DNA against nuclease degradation (Borchard, 2001). Cationic polymers such as chitosan are used to condense and deliver DNA both *in vitro* and *in vivo* (Kim et al., 2007). Biocompatibility, low immunogenicity and minimal cytotoxicity render chitosan NPs (100–250 nm) a good alternative to viral or lipid mediated transfection (Mao et al., 2001).

The discovery of RNA-based silencing has given new vision to crop improvement by allowing specific control of insect pests that feed upon the double stranded RNA (dsRNA) producing plants (Auer and Frederick, 2009; Price and Gatehouse, 2008). RNA interference (RNAi), first described in the worm *Caenorhabditis elegans*, resulted in degradation of homologous mRNA on the introduction of long dsRNA in cells. RNAi-mediated gene knockdown was reported in several insect orders, including Diptera, Coleoptera, Hymenoptera, Orthoptera, Blattodea, Lepidoptera and Hemiptera (Araujo et al., 2006; Baum et al., 2007; Huvenne and Smagghe, 2010; Mao et al., 2007; Timmons and Fire, 1998; Tomoyasu and Denell, 2004; Turner et al., 2006). The delivery of dsRNA in these studies was by microinjection into the insect hemocoel, whereas in *C. elegans*, feeding of bacteria expressing dsRNA or even soaking in dsRNA solution was followed, that is not practical for field application (Tabara et al., 1998;Timmons et al., 2001; Tomoyasu and Denell, 2004). Production of sufficient dsRNA by transgenic plants and its delivery in a sufficiently undegraded state to the insect to achieve control required continuous administration of high levels of dsRNA, due to degradation of dsRNA in the insect gut (Baum et al., 2007; Mao et al., 2007). Chitosan NPs (<500 nm) with entrapped siRNA performed well as vectors for siRNA delivery vehicles (Katas and Alpar, 2006). Recently, Zhang et al. (2010) demonstrated that chitosan NPs (100–200 nm) stabilized and delivered dsRNA (against chitin synthase genes), to mosquito larvae by feeding that resulted in increased larval susceptibilities, in presence of chitin synthase inhibitors (Table 1). Chitosan NPs could prove to be efficient in dsRNA delivery due to their efficient binding with RNA, protection and the ability to penetrate through the cell membrane. NPs based siRNA formulations may contribute to insect pest control while avoiding the lengthy process of plant transformations.

4. Nanosensors in agriculture

Nanotechnology based sensor systems for applications in agriculture are still at the basic research level. Development of sensors and diagnostic devices for on-site monitoring will allow farmers to closely monitor environmental conditions, for plant growth and protection. These detection systems can contribute to increased productivity and decreased the use of agrochemicals (*e.g.* antibiotics, pesticides, nutrients) by early intervention.

4.1. Nanobiosensors for pesticide detection

Several studies were conducted for development of analytical tools for pesticide residue determination. In parallel with typical chromatography, immunochemical assays based on bio-molecules were employed as an alternative for pesticide measurement by virtue of its high selectivity, sensitivity, and reliability as well as its rapidity (Gabaldon et al., 1999). Traditional immunoassay is performed as discrete tests, that is, one assay for one analyte and several detecting runs for all of the components in a complex system. Application of nanomaterials adds further advantages to such systems, such as: further miniaturization, measurement of more variables, greater sensitivity, less sample material required, faster detection rates, read-outs in real time and application of novel detection methodologies (*e.g.* electronic, colorimetric, fluorometric, and mass changes). Currently uni-molecular and array type of nanomaterial based biosensors are being developed for detection of pesticides (Table 1). However, the format of bio-sensors varies, from free biomolecules to those conjugated to a substrate such as NPs, nanowires, nanotubes and thin-films. Interaction of the target with the biosensor can be measured either directly or indirectly by recording the changes in color, fluorescence or electrical potential. In array technologies, multiple biomolecules are fixed to a substrate allowing multiple analytes to be measured simultaneously.

Among the uni-molecular array systems a gold NPs (30 nm) based dipstick competitive immuno-assay with sensitivity of 27 ng ml^{-1} was developed to detect organochlorine pesticide such as DDT (Table 1, Lisa et al., 2009). Gold NPs have the property of agglomeration associated with color production that was used for pesticide detection. Development of a color signal aided easy visual detected when gold NPs labeled antibodies bound to the pesticide residues. The gold NP based dipstick technique was suitable for the detection of several toxins in food and environmental samples and can be applied for rapid on-site testing of pesticides (Lisa et al., 2009). Vinayaka et al. (2009) used cadmium telluride quantum dots (CdTe QDs), semiconductor fluorescent NPs, in a fluoroimmunoassay to detect 2, 4-dichlorophenoxyacetic acid (2, 4-D), a herbicide. It was possible to detect 2,4-D up to 250 pg l^{-1}. Wang et al. (2009) developed a zirconium oxide NPs (~50 nm) based immunoassay for sensitive detection (0.02 nM) of organophosphate pesticides using phosphorylated enzyme acetylcholinesterase as a potential biomarker (Table 1). Joshi et al. (2005) developed a disposable, sensitive biosensor for organophosphorus pesticides (0.5 nM) based on acetylcholinesterase binding on multi-wall carbon nanotubes modified thick film strip electrode (Table 1). The biosensor reproducibily detected low paraoxon at parts per billion (0.145) levels. Gan et al. (2010) also developed an acetylcholinesterase coated iron oxide magnetic NPs (30 nm) bound carbon nanotubes and zirconium oxide NPs (31.5 nm) composite on the screen printed electrode surface for detection of dimethoate, an organophosphorus pesticide (Table 1). The sensitivity of the biosensor was at 50 pg l^{-1}. An enzyme based organophosphate array biosensor was developed by Ramanathan et al. (2009). Organophosphate hydrolase (OPH) was conjugated to a pH-responsive fluorophore encapsulated in silica NPs (100–500 nm) and patterned to a waveguide surface (Table 1). Silica NPs-encapsulated OPH was stable for nearly 60 days and retained a detection limit (34 μM) of parts per million for paraoxon. The ability to integrate enzymes for catalytic detection with the capability to detect chemical agents may potentially extend the versatility of the array nano-biosensor to both biological and chemical contaminants.

Kaushik et al. (2009) fabricated a nucleic acid sensor via immobilization of single standard calf thymus deoxyribose nucleic acid onto a chitosan nanobiocomposite film containing iron oxide NPs (Fe_3O_4, 22 nm), deposited onto indium-tin-oxide coated glass surface for pyrethroid, cypermethrin and permethrin, detection (Table 1). This disposable nanobiocomposite bioelectrode was stable for about two months under refrigerated conditions and detected cypermethrin and permethrin rapidly (>50 s) at 0.0025 ppm (Kaushik et al., 2009).

The widespread use of various pesticides and their mixtures in the agricultural fields causes the multiresidue retention problem in the environment. Thus, it is necessary to simultaneously identify several pesticides in a complex sample. By comparison, multianalyte immunoassay that can simultaneously discriminate at least two analytes in a single run displays noticeable advantages such as high sample throughput, attractive assay efficiency, and low consumption of reagent, labor and time (Fu et al., 2007; Kricka, 1992). Guo et al. (2009) developed a gold NPs (40 nm) based portable one-step strip assay allowing simultaneous screening for two pesticides carbofuran and triazophos within a short time (8–10 min) without any equipment (Table 1). A bispecific monoclonal antibody (McAb), against both carbofuran and triazophos, was labeled with nanogold in comparison to the nanogold labeled anti-carbofuran McAb and anti-triazophos McAb as detector reagents. Analysis of spiked water samples, by visual comparison of pesticide standard tests between the two formats, indicated that the detection limit was at parts per million for carbofuran (32 μg l^{-1}) and triazophos (4 μg l^{-1}) (Guo et al., 2009).

The scope for application of nanotechnology for the development of sensors that are specific, fast, handy and inexpensive is vast and the key challenge would be the commercialization of biosensors. Furthermore, combining nanotechnology with microfluidic devices, that allow flow of micro-volumes of liquid through channels, will promote further miniaturization and rapid sampling. Cantilever nanomechanical devices, with springboard like-mechanism, are interesting as they detect the presence of specific target molecules, when the target binds a reporter molecule attached to the cantilever. Cantilever arrays can give rise to a sensitive nanosensor electronic 'nose' or 'tongue'. The future would see the development of multianalyte array sensors, microfluidic devices and cantilever arrays to make environmental monitoring easy.

4.2. Nanobiosensors for plant pathogen detection

Currently, several methods of microbial identification and typing are available for most plant pathogens. Traditional culture based methods are time consuming (Fletcher et al., 2006). Techniques based on biochemical profiles for identification are limited by the population of characterized strains in the databases. Sensitive and specific serological techniques such as ELISA and indirect fluorescent antibody staining depend on the titer and specificity of the antibody either monoclonal or polyclonal (Lister 1978; Uddin et al., 2003, Yuen et al., 1998). Cross-reactivity among closely related strains prevents their clear identification (Cho and Goodman, 1979: Jain et al., 1992). Costly, nucleic-acid based polymerase chain reaction methods such as restriction fragment length polymorphism, DNA fingerprinting and amplification of the internal transcribed spacer region from rRNA gene increase specificity of identification (Bariana et al., 1994; Doorn et al., 2007; Gao et al., 2004; Schaad et al., 2002).

A novel microbial detection technology based on NPs is being developed. Silica-based NPs (60 nm) were filled with a fluorescent dye and conjugated to an antibody specific to a surface antigen of the microbe of interest (Zhao et al., 2004). Detection of a single bacterial cell was possible using this technique (Zhao et al., 2004). This method has potential for sensitive detection of plant pathogens.

5. Pesticide degradation

Pesticide-contaminated soil and water treatment ranges from conventional methods such as incineration, phytoremediation, and photochemical processes to innovative methods such as ultrasound-promoted remediation and other advanced oxidation processes (Bhatkhande et al., 2001, Chaudhry et al., 2002; Farre et al., 2007; Hee Joo and Cheng, 2006; Hoffmann et al., 1995). Degradation of bio-recalcitrant pollutants using NPs is another promising approach (Hee Joo and Cheng, 2006; Zhang, 2003). Recent studies showed that pesticides such as atrazine, molinate, and chlorpyrifos are susceptible to degradation with nanosized zerovalent iron (ZVI,1–100 nm). Cyclodiene insecticides such as endosulfan, however, were generally resistant (Hee Joo and Cheng, 2006). Nanosized ZVI had a greater reactivity than granular ZVI, and their direct injection into the groundwater plume to minimize installation costs, was suggested (Zhang, 2003). Applications of ZVI structures and iron oxide NPs may be for removal of humic material and toxins (Giasuddin et al., 2007; Waychunas et al., 2005). However, little is known about the long-term performance of these nanoparticles/colloidal systems. The application of NPs such as biopolymer stabilized FeS (200 nm), are in scavenging and degradation of lindane, a persistent organic pollutant found in drinking water as well as in food (Table 1, Paknikar et al., 2005). Other approaches such as photocatalytic decomposition of pesticide residues using titania doped with Fe_2O_3 or other metals sprayed directly on crops or even incorporated into the pesticide formulation are promising (Sasson et al., 2007). Layer-by-layer surface (LbL) nano-engineering is a novel strategy for direct surface modification of colloidal entities, which utilizes sequential adsorption of oppositely charged polyelectrolytes to form a complex assembly via electrostatic interactions (Yang et al., 2005). Guan et al. (2008) directly encapsulated microcrystals of the insecticide imidacloprid (IMI) by LbL assembly using polysaccharides chitosan and sodium alginate followed by addition of addition of photocatalytic NPs (Table 1). Photocatalytic degradation and mineralization of the IMI by TiO_2 NPs (~30 nm) and silver and sodium dodecyl sulfate modified TiO_2 NPs were reported.

6. Soil structure and remediation

Clays are sub-micrometric soil particles. Common clays are layered phyllosilicate materials, with a polymeric silicate base, which are nanodimensional in one plane. Transmission electron (TEM) and high resolution transmission electron (HRTEM) microscopy showed that clays are composed of stacked tetrahedral and octahedral sheets (Wilson et al., 2008). Other inorganic nanomaterials such as the tubular aluminosilicate imogolite and its non-tubular precursor called proto-imogolite are soil components (Farmer et al., 1983). NPs such as iron and silica originate from natural weathering of bedrocks. Other naturally occurring NPs are iron oxides (2–5 nm length), as colloidal phases of ferrihydrite, associated with organic matter in river-borne material (Allard et al., 2004). However, Yang and Watts (2005) showed that alumina NPs (aluminum oxide) are detrimental to plant root growth.

Remediation is another aspect of the role of NPs in soil structure. In 2003, a nanotechnology based soil binder, a type of quick-setting organic and biodegradable mulch, was developed by a US based company (*ETC* Group, 2004). The reaction caused silicates in the soil and silicates in the product to self-assemble into a kind of crust that remained up to a year. The crust was claimed to prevent soil runoff and allowed seeds blended into the product to germinate. It was sprayed over 1400 acres of Encebado mountain in New Mexico to prevent erosion following forest fires, as well as on smaller areas of forest burns in Mendecino County, California.

Bioremediation of toxic metals is an important aspect that affects plant growth. Copper, as a free ion catalyzes the production of damaging radicals. Thus, all life forms attempt to prevent copper toxicity. Plants diminish excess copper in two structural regions: in sub-aerial tissues by rare hyperaccumulators, that bind cationic copper to organic ligands; and in roots by metal tolerant plants that segregate copper by similar mechanisms. The common wetland plants or the peats *Phragmites australis* and *Iris pseudoacorus* transform copper into metallic NPs in and around their roots with assistance from endomycorrhizal fungi when grown in contaminated soil (Manceau et al., 2008). Biomolecular responses to oxidative stress were responsible for the NP formation. Peat cultivation can contribute to copper biorecycling and rhizosphere containment to prevent copper biomagnification.

7. Future of farming- nanobio-farming?

It is important to note that nanomaterials, owing to their increased contact surface area, might have toxic effects that are not apparent in the bulk materials especially in open agricultural ecosystems (Nel et al., 2006). The selection of nanomaterial for application in the field may be critical as materials which are non-toxic, biocompatible and biodegradable are desirable. Few food and nutrition products that contain nanoscale additives already in the market, such as iron in nutritional drink mixes, micelles that carry vitamins, minerals and phytochemicals in oil and zinc oxide in breakfast cereals (Hoyt and Mason, 2008).

The future if foreseen would allow the advancements in agricultural nanotechnology to promote 'precision farming' allowing optimum use of the natural resources with judicious farming practices. Different sensor and controlled delivery technologies would change the face of farming. The use of a network of sensors, global positioning system, global information system and actuators throughout an agricultural area could measure (data and statistics) and report on a number of different environmental, crop and pest variables. These reports would support the experience of the farmer and permit choices for intervention during irrigation, fertilization, pest control and even harvesting. Although costly, this would be largely offset by the rising cost of food, the need for higher quality and increasing legislation. The technology already exists to measure each of these variables. However, measurement requires technical expertise, is labor-intensive and can take days, by which time the opportunity for optimal intervention could be missed. By providing robust, portable or remote *in situ* nanotechnology based sensing and monitoring, backed up with analytical software, farmers can begin to make their own informed choices, in real-time, and apply agrochemicals or engage expert help only when necessary.

8. Conclusion

In the current agricultural scenario, the extensive use of agrochemicals to boost agricultural production has polluted not only the top soil, but also groundwater. Increasing agricultural productivity is necessary, but keeping the in mind the damage to the ecosystem new approaches need to be considered. Nanotechnology is becoming increasingly important for the agricultural sector. Promising results and applications are already being developed in the areas of delivery of pesticides, biopesticides, fertilizers and genetic material for plant transformation. The use of nanomaterials for delivery of pesticides and fertilizers is expected to reduce the dosage and ensure controlled slow delivery. A main contribution anticipated, is the application of nanoparticles to stabilize biocontrol preparations that will go a long way in reducing the environmental hazard. A major hurdle in the removal of harmful contaminants from soil was its detection in the field, which was costly with conventional methods. Nanotechnology, by exploiting the unique properties of nanomaterials, has developed nanosensors capable of detecting pathogens at levels as low as parts per billion. Apart from detection, nanotechnology also has solutions for degrading persistent chemicals into harmless and sometimes useful components. Agricultural technology should take advantage of the powerful tools of nanotechnology for the benefit of mankind. The tools of nanotechnology can be employed to address the urgent issues of environmental protection and pollution. Nanotechnology can endeavor to provide and fundamentally streamline the technologies currently used in environmental detection, sensing and remediation.

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