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Development and efficacy evaluation of novel adhesive pesticide nano-delivery systems

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Résumé

Changjiao SUN. (2019). Développement et évaluation de l'efficacité de nouveaux systèmes de nanoparticulation de pesticides adhésifs (Thèse de doctorat en anglais). Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège. 130 pages, 13 tableaux, 27 figures.

Résumé- La mise au point de nouveaux pesticides respectueux de l'environnement est à la base de la prévention des grandes catastrophes biologiques et de la sécurité alimentaire. Cependant, il a été estimé que 70% à 90% des pesticides appliqués sont soit perdus dans l'air, soit lessivés, ce qui a provoqué de nombreux effets néfastes, tels que la résistance des organismes nuisibles, le risque pour la santé humaine, la toxicité pour les organismes non ciblés, ainsi que la contamination de l'environnement. Au cours de la dernière décennie, la mise au point de formulations de nanopesticides a démontré son grand potentiel en vue d'améliorer les performances des pesticides en construisant des systèmes de nanotransporteurs. De plus, en termes de microstructure du feuillage des cultures, la surface des nanoparticules peut être facilement modifiée par des groupes d'affinité afin d'améliorer l'adhésion et réduire les pertes de ces feuillages de cultures.

Dans cette thèse de doctorat, nous visions à développer de nouvelles formulations d'avermectine. Afin de réduire la pollution par les solvants organiques et de prévenir la dégradation prématurée de l'avermectine, une libération stable et contrôlée de formulations de pesticides à forte affinité pour le feuillage des cultures et une longue durée de rétention sur ces feuillages ont été construites pour augmenter le taux d'utilisation efficace des pesticides et réduire les pertes sur l'environnement. Et l'efficacité des nanoformulations a également été évaluée sur ravageurs et espèces non cibles.

Tout d'abord, le charbon actif mésoporeux modifié par un agent tensioactif (MAC) a été utilisé pour absorber l'Av afin d'améliorer sa photo stabilité et permettre la libération prolongée d'avermectine. Les résultats suggèrent que le MAC modifié au dodécyl sulfate de sodium (SDS) avait une excellente absorption de l'avermectine et que l'absorption pourrait être représentée par le modèle isotherme de Langmuir. Le système d'administration Av-MAC-SDS a significativement amélioré la libération prolongée d'avermectine et également inhibé efficacement la photo-dégradation de l'avermectine.

Ensuite, la biocompatibilité et l'acide polylactique biodégradable (PLA) ont été utilisés comme matériau de support. L'acide tannique (TA), une molécule naturelle bioadhésive, a été appliqué pour modifier les systèmes de nano-délivrance d'abamectine. Les nanoparticules ont montré une excellente libération prolongée continue et une photo-stabilité. Par rapport aux nanopesticides non modifiés, le taux de rétention des nanoparticules modifiées sur le feuillage a été remarquablement accru de plus de 50% et la toxicité intérieure par la méthode de trempage contre *Myzus persicae* a également été augmentée

Enfin, l'activité insecticide de l'abamectine nano-formulée à base de PLA a été examinée sur le puceron du pois, *Acyrthosiphon pisum* (Hemiptera: Aphididae), et sur le prédateur de puceron *Adalia bipunctata* (Coleoptera: Coccinellidae). Une tour Potter de pulvérisation de laboratoire de précision a été utilisée pour effectuer des essais toxicologique par application directe en laboratoire. Un effet insecticide comparable à la nanoformulation modifiée au TA a été observé par rapport au concentré émulsifiable commercial (CE) contre le puceron. Les nanoformulations ont démontré une toxicité plus faible pour les coccinelles non ciblées.

Ces résultats devraient être bénéfiques pour la mise au point de nouveaux nanopesticides adhésifs aux feuilles ayant une durée de rétention et une biodisponibilité élevées.

Mots clés: nanopesticides; formulation; adhésif; l'avermectine

Abstract

Changjiao SUN. (2019). Development and efficacy evaluation of novel adhesive pesticide nano-delivery systems. (PhD Dissertation in English). Gembloux, Belgium, Gembloux Agro-Bio Tech, Université de Liège. 130 pages, 13 tables, 27 figures.

Abstract- Developing new eco-friendly pesticides is the foundation for preventing major biological disasters and ensuring food security. However, it has been estimated that 70% to 90% of the applied pesticides are either lost in the air or run-off, which has caused many adverse effects, such as pest resistance, risk to humans and non-target organisms and environmental contamination. In the recent decade, the development of nanopesticide formulations has shown a great potential to improve the performance of pesticides by constructing nano-delivery systems. Moreover, in terms of the crop foliage microstructure, the surface of nanoparticles can be easily modified by affinity groups to improve adhesion and decrease the loss from crop foliage.

In this PhD thesis, we aimed at developing new formulations of avermectin (Av). In order to decrease the organic solvent pollution and prevent premature degradation of avermectin, stable and controlled release of pesticide, formulations with high affinity for crop foliage and long retention time on crop foliage were constructed to increase the effective utilization rate of pesticides and minimize loss to the environment. The efficacy of the nanoformulations was evaluated as well on pests and non-target species.

First, surfactant-modified mesoporous activated carbon (MAC) was employed to absorb Av in order to improve its photo-stability and allow for sustained release of avermectin. Sodium dodecyl sulfate (SDS) modified MAC had excellent absorption of avermectin, and the absorption could be represented by the Langmuir isotherm model. The Av-MAC-SDS delivery system significantly improved sustained release of avermectin and also effectively inhibited the photo-degradation of avermectin.

Then, biocompatibility and biodegradable polylactic acid (PLA) was employed as the carrier material. Tannic acid (TA), a bioadhesive natural molecule, was applied to modify abamectin nano-delivery systems to enhance retention time on foliage. The nanoparticles showed excellent continuous sustained release and photo-stability. Compared with unmodified nanopesticides, the retention rate of modified nanoparticles on the foliage was remarkably enhanced by more than 50% and indoor toxicity with dipping method against *Myzus persicae* L. was also increased.

Finally, insecticidal activity of PLA-based nano-formulated abamectin was examined on the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae), and the aphid predator *Adalia bipunctata* (Coleoptera: Coccinellidae). A Potter Precision Laboratory Spray Tower was used to conduct direct spray laboratory bioassays. A comparable insecticidal effect of TA modified nanoformulation was observed compared to commercial emulsifiable concentrate (EC) against the aphid. And the nanoformulations had lower stomach toxicity on non-target lady beetles.

These results are expected to be beneficial to develop novel leaf-adhesive nanopesticides with high retention time and bioavailability.

Keywords: nanopeticides; formulation; adhesive; avermectin

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Changjiao Sun

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abam	Abamectin
AI	Active ingredient
ALC	Abamectin loading content
Av	Avermectin
Az	Azidobenzaldehyde
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CA	Contact Angle
CMC	Critical micellar concentration
CMCS	Carboxymethyl chitosan
DEPA	Diethylphenylacetamide
DESAUN	Department of Economic and Social Affairs of the United Nations
DLS	Dynamic light scattering
EB	Emamectin benzoate
EC	Emulsifiable concentrate
EO	Essential oil
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization
FOCUS	FOrum for the Co-ordination of pesticide fate models and their Use
HPLC	High performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
IPM	Integrated Pest Management
LC	Lambda-Cyhalothrin
LC_{50}	Lethal concentration 50%
LMMG	Low-molecular mass gelator
MAC	Mesoporous activated carbon
MWCO	Molecular weight cut off
NS	Nanosphere
PCA	Poly (citric acid)
PCL	Polycaprolactone
PDI	Polydispersity index
PEG	Polyethylene glycol
PEO	Eolyethylene oxide
PGA	Polyglycolic acid
PHB	Polyhydroxybutyrate
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PMMA	Poly (methyl methacrylate)
PSD	Particle size distribution

PVA	Poly(vinyl alcohol)
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
TA	Tannic acid
TBAB	Tetrabutylammonium bromide
TEM	Transmission electron microscope
UAV	Unmanned aerial vehicles
USFDA	US Food and Drug Administration
UV	Ultraviolet
WDG	Water-dispersible granules
λ-Cy	Lambda-cyhalothrin

1

General introduction

Pesticides play an important role in controlling biological disasters and increasing the crop yields. It is estimated that more than 30% of total output of agricultural products all over the world has been restored due to the application of pesticides. Most of the active ingredients of pesticide are insoluble in water, which need to be added with organic solvent, emulsifier and other auxiliary ingredients and processed into a suitable formulation to facilitate the spray application in the field. However, inefficient usage of conventional pesticide formulations caused by the off-target loss is a crucial problem. It was estimated that the loss and decomposition rate of pesticide on crop foliar is up to 70%, caused by spray drift, run-off and rolling down during field application, which has caused many adverse effects, such as pest resistance, risk to humans and non-target organisms and environmental pollution. In order to avoid the deleterious effects of pesticides, the efforts of agrochemical industry are not only focused on looking for new active substances, but also in developing new pesticide formulations.

Nanotechnology and nanomaterial own a promising future in sustainable agriculture development. Due to the unique properties, such as small size, chemical composition, surface structure, solubility, shape and aggregations, it shows great potential to formulate nano-based smart pesticides formulations for alleviation of problems mentioned above. The key motivation to develop new formulations is to improve the efficacy of pesticides, while lowering doses and application frequency by regular, precise, long and targeted delivery. Nanopesticides could be developed by two pathways: directly processing into nanoparticles (nanosized pesticides) and loading pesticides with nanocarriers to form delivery systems. Research work relative to nanopesticide delivery systems can be roughly divided into two categories, one deals with the efficacy and fate of the developed nanopesticides, and the other explores new nanoformulations with expected functions.

Recently, development of novel adhesive nanopesticides gradually becomes a hotspot. According to the crop foliage microstructure, the surface of nanopesticides can be modified with affinity groups to improve adhesion and decrease the loss from crop foliage caused by scattering or rolling off. Natural adhesive materials such as plydopamine has been intensively studied as the adhesive coating of pesticide. However, the extraction process of dopamine is complicated and costly. As an alternative, a natural polyphenol, tannic acid that can be extracted from various plants with low cost is more practical for application as the adhesive coating of nanopesticides.

In this thesis, we reviewed the development strategies and prospects of nano-based smart pesticide formulation, as well as current developments, environmental risks and future challenges of the polymer-based nanoinsecticides. Then, surfactant-modified mesoporous activated carbon was employed to absorb avermectin in order to improve its photostability and allow for sustained release of avermectin. Besides, tannic acid-modified abamectin nano-delivery systems were constructed and the properties of the adhesive nanopesticide were characterized. Finally, in order to gain a comprehensive efficacy evaluation of the novel formulation, we examined the insecticidal activity of the adhesive nanopesticide on the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae), and the aphid predator *Adalia bipunctata* (Coleoptera: Coccinellidae). This work could contribute to the development of organic solvent free and high efficacy nanopesticide formulation.

2

Bibliographic introduction

From Zhao, X., Cui, H., Wang, Y., Sun, C., Cui, Bo., Zeng, Z., 2018. Development Strategies and Prospects of Nano-based Smart Pesticide Formulation. J. Agric. Food Chem. 66, 6504-6512.

From Sun, C., Zeng, Z., Cui, H., Verheggen, F., 2019. Polymer-based nanoinsecticide: Current developments, environmental risks and future challenges. Submitted to BASE.

Pesticides are vital in agriculture to defend against biological disasters, ensure crop productivity and sustain steady growth of the crop yields. However, the inefficient usage of pesticides results in serious pollutants in soil and water systems, chemical residues in crops and food products, which is a potential threat to human health. Nanotechnology provides a new strategy for constructing new pesticide formulations, which are beneficial to sustainable agriculture development. Due to the unique properties of nanomaterials, such as small size, big surface area, and easy modification of surface groups, it shows great potential to improve the dispersion, stability, duration and efficacy of pesticides by preparing nano-based formulations. In this chapter, we discuss the advantages of nano-based formulations, as well the challenges and scientific issues for improvement of pesticide efficacy and safety.

2.1 Development strategies and prospects of nano-based smart pesticide formulation

Abstract: Pesticides are important inputs for enhancing crop productivity and preventing major biological disasters. However, more than 90% of pesticides run off into the environment and reside in agricultural products in the process of application as a result of the disadvantages of conventional pesticide formulation, such as the use of a harmful solvent, poor dispersion, dust drift, etc. In recent years, using nanotechnology to create novel formulations has shown great potential in improving the efficacy and safety of pesticides. The development of nano-based pesticide formulation aims at precise release of necessary and sufficient amounts of their active ingredients in responding to environmental triggers and biological demands through controlled release mechanisms. This paper discusses several scientific issues and strategies regarding the development of nano-based pesticide formulations: (i) construction of water-based dispersion pesticide nanoformulation, (ii) mechanism on leaftargeted deposition and dose transfer of pesticide nanodelivery system, (iii) mechanism on increased bioavailability of nanobased pesticide formulation, and (iv) impacts of nanoformulation on natural degradation and biosafety of pesticide residues.

Keywords: nanoformulation, pesticide, nanotechnology, agriculture, nanodelivery system

1 Introduction

One of the global challenges faced by the agriculture sector is the sustainable food production for the rapidly growing human population to 9.7 billion by the 2050 (DESA/UN, 2015; Godfray et al., 2014). Therefore, pesticides and fertilizers are indispensable to maximize the agricultural productivity (De Oliveira et al., 2014). Generally, Pesticides are vital in agriculture to defend against biological disasters, ensure crop productivity and sustain steady growth of the crop yields (EPA, 2007). According to the statistics of Food and Agriculture Organization of the United Nations (FAO), pest and pathogen control with pesticides has restored 30% of total output of agricultural products all over the world (FAO, 2007; Lamberth et al., 2013). Despite their benefit in agriculture, extreme dependency on pesticides results in redundant usage. It has been estimated that the annual input amounts of pesticides have reached 4.6 million tons worldwide and more than 90% of the applied pesticides are either lost to the air or run-off (Ghormade et al., 2011; Perlatti et al., 2013), which has caused many adverse effects, such as pest resistance, risk to humans and non-target organisms and environmental contamination (Dawkar et al., 2013; Kohler et al., 2013; Talebi et al., 2011) (Figure 1).

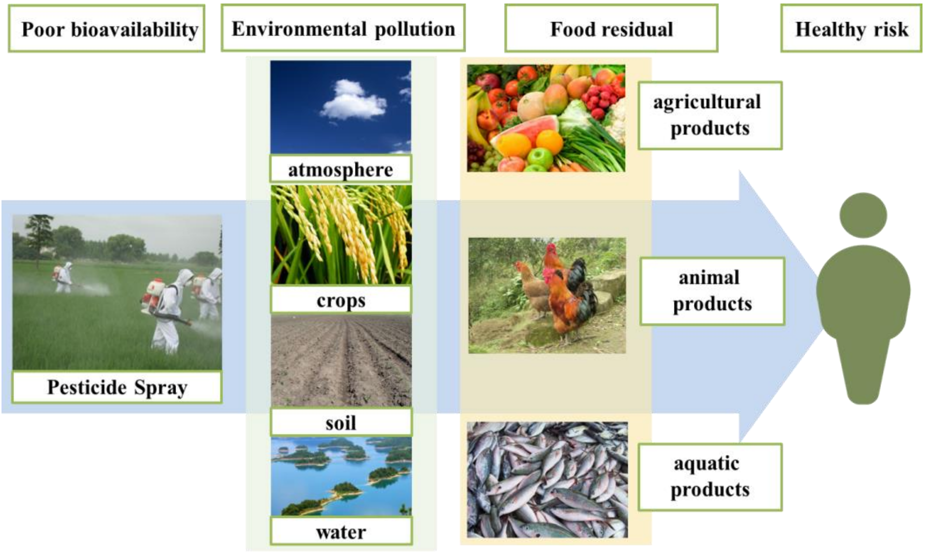

Figure 1. Inefficient use of pesticides causes a series of environmental problems

Most of the active ingredients (AIs) of pesticide are water-insoluble organic compounds, which need to be added with carrier, solvent, emulsifier, dispersant, and other auxiliary ingredients and processed into a suitable formulation to facilitate the spray application in the field (Ghormade et al., 2011). However, the off-target loss is a crucial problem for inefficient usage of conventional pesticide formulations during the application. It was estimated that the loss and decomposition rate of pesticide on crop foliar is typically up to 70%, caused by spray drift, runoff and rolling down during field application (Song et al., 2017; Nuruzzaman et al., 2016), and the actual utilization of biological target uptake is less than 0.1% after dust drift and rainwater leaching (Massinon et al., 2017; He et al., 2016) (Figure 2). The inefficient usage of pesticides results in serious pollutants in soil and water systems, chemical residues in crops and food products, which is a potential threat to human health (Hayles et al., 2017). These environmental problems and health risks have aroused universal concerns.

Nanotechnology has great potential in sustainable agriculture development; thus, using nanotechnology to formulate nano-based smart formulations for pesticides by virtue of nanomaterial-related properties has shown great potential for alleviation of these problems (Hamburg et al., 2012; Morris et al., 2011; Scott et al., 2012). Nano-based smart formulations could release their AIs in response to environmental triggers and biological demands more precisely through targeted delivery or controlled release mechanisms. Developing new advanced nano-based formulations that remain stable and active in the spray condition (sun, heat, and rain), penetrate and deliver to the target, prolong the effective duration, and reduce the runoff in the environment is one of the hotspots in the field of agricultural applications of nanotechnology (Ghormade et al.; Smith et al., 2008; ObservatoryNANO, 2010). In recent years, European Commission and the United States Environmental Protection Agency (U.S. EPA) have successively enacted rules on the management and usage of nanopesticides (FAO/WHO, 2009; U.S. EPA, 2011).Bayer, DuPont, Syngenta, and other agrochemical enterprises have also paid great attention to the development of nano-based pesticide formulations, and some products has been applied to crop production or plant protection (FAO, 2010).

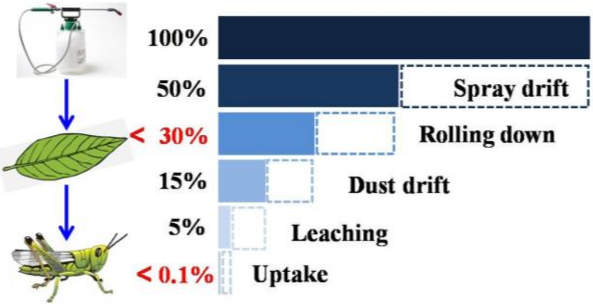

Figure 2. Low efficiency of conventional pesticide formulations

2 Nano-based pesticide formulation: properties and advantages

Nanotechnology involves the manufacture, manipulation, and application of materials that have at least one size dimension at the nanometer (1-100 nm) range (Auffan et al., 2009). Particles exhibit special properties, such as size-dependent qualities, high surface/volume ratio and unique optical properties at a critical length scale of less than 100 nm (Ghormade et al., 2011). However, because other phenomena (transparency, turbidity, stable dispersion, etc.) that extend the upper limit are occasionally considered, a broader definition of nano-based pesticide formulations is accepted because systems with dimensions smaller than 1000 nm have novel properties associated with their small size (Morris et al., 2011; Kah et al., 2013; Kah et al., 2014).

Nanomaterials have great promise regarding their application in nano-based pesticide formulation due to their small size, big surface area, and target modified properties. Nano-based formulation may bring beneficial improvements in properties and behaviors of pesticides, such as solubility, dispersion, stability, mobility, and targeting delivery. Furthermore, it might significantly improve the efficacy, safety, and economic effects of traditional pesticides by increasing efficacy, extending effect duration, reducing the dose required, providing capability to control the release of AIs, and improving stability of payloads from the environment, subsequently diminishing runoff and environmental residuals (Figure 3).

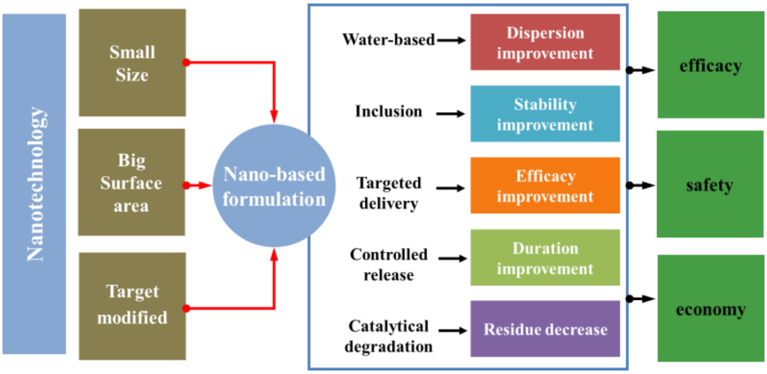

Figure 3. Nano-based formulation brings beneficial improvements in pesticide properties

The size, shape, surface charge, crystal phase and presence of different modified functional groups of nanoparticles are critical factors in their application (Kah et al., 2013; Zhao et al., 2014). A broad variety of natural or synthesized materials can be used in construction of pesticide nanoformulations, such as metals, metal oxides, non-metal oxides, carbon, silicates, ceramics, clays, layered double hydroxides, polymers, lipids, dendrimers, proteins, quantum dots, etc (Oskam et al., 2006; Perez-de-Luque et al., 2009; Gogos et al., 2012; Khot et al., 2012). Nanopesticides may be developed by two pathways: directly processing into nanoparticles (nanosized pesticides) and loading pesticides with nanocarriers in delivery systems (Ghormade et al., 2011). In nanocarrier systems, pesticides are encapsulated inside the nanoparticulate polymeric shell, absorpted onto the nanoparticle surface, attached on the nanoparticle core via ligands, or entrapped within the polymeric matrix (Figure 4).

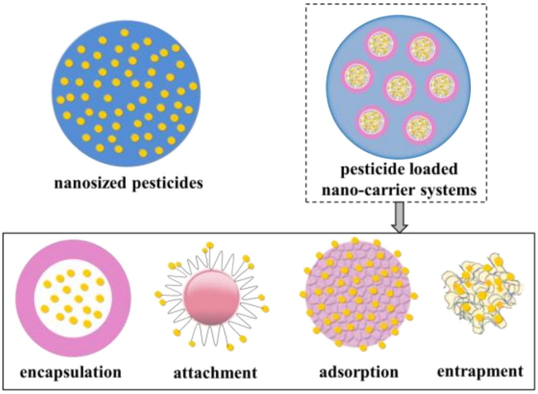

Figure 4. Schematic diagram of nano-based pesticide formulation

A variety types of nanoformulation have been developed, including nanoemulsions, nanocapsules, nanospheres, nanosuspensions, solid lipid nanoparticles, mesoporous nanoparticles, and nanoclays (Ao et al., 2013; Wang et al., 2007; Puoci et al., 2008; Frederiksen et al., 2003; Guan et al., 2010). Aqueous nanoemulsion and nanosuspension of pesticides increase solubility of waterinsoluble AIs, eliminate the toxic organic solvents, and would gradually substitute the conventionally EC products (Zhang et al., 2008; Rabinow, 2004; Liu et al., 2011). Nanocapsule and nanosphere are suggested as vehicles for the environmentally sensitive pesticides as a result of their capability to slow the release of AIs, improve stability of formulation, prevent early degradation, and extend the longevity of pesticides (Shang et al., 2006; Liu et al., 2002; Boehm et al., 2003; Qian et al., 2011). Mesoporous nanoparticles, such as nanoclay, activated carbon, and porous hollow silica, are also verified to be suitable for the controlled release and delivery systems for the water-soluble and fat-dispersible pesticides, which possess high drug-loading capacity, good biocompatibility, low toxicity, and multistage release pattern (Wang et al., 2012; Li et al., 2006).

3 Challenges and scientific issues

The mode of pesticide application influences their efficiency and environmental impact (Ihsan et al., 2007; Matthews, 2008; Matthews et al., 2000). Insect pests and pathogens are the targets of pesticides. However, it is extremely difficult to directly spray the pesticides on pests or pathogens. As a result, the pesticides are sprayed on the crop foliage to form an effective toxic zone, maintaining the toxic stress on the pests or pathogens. Currently, the spraying system of pesticide application needs to focus on efficacy enhancement and spray drift management (Ghormade et al., 2011).

Most of the pesticide AIs are poorly soluble in water. One of the challenges associated with pesticide formulation is increasing their solubility and dispersion in aqueous solution. In addition, most crop leaf surfaces are highly hydrophobic, which inhibits liquid deposition (Neinhuis et al., 1997; Burton et al., 2006). Thus, another challenge is reducing the spray drift and runoff loss on the hydrophobic foliage. As shown in Figure 5, downsize of pesticide particles benefits to significantly improve their water dispersion, targeting coverage and insecticidal activity as a result of the smaller particle size and higher surface area. In addition, pesticide nanoformulations increase adhesion and deposition of droplets on the leaves through leaf-affinity modification.

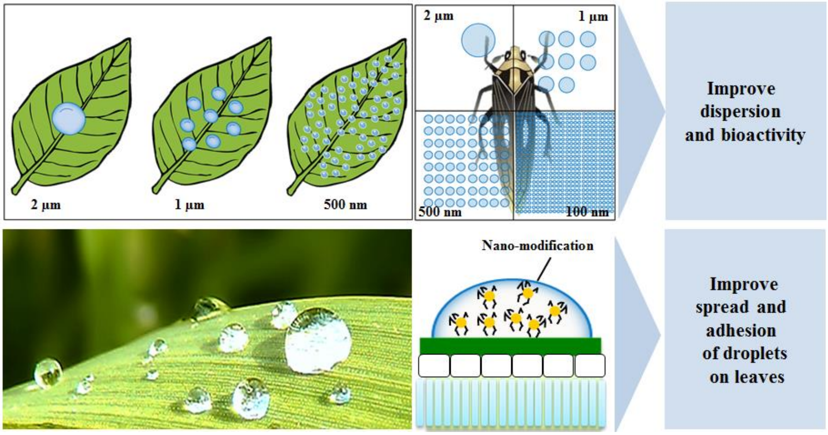

Figure 5. Downsize of pesticides increases bioavailability and efficiency

After spraying on the foliage, the pesticide droplets spread and adhere on the leaf surfaces, and then the AIs deposit, release, and transfer from the foliage to the pest or pathogen targets and finally kill the insects or pathogens before degradation (Figure 6). Therefore, water dispersion, leaf affinity, bioavailability, and residue degradation are the most critical factors regarding development of nano-based pesticide formulations. Four key scientific issues for improvement of pesticide efficacy and safety are proposed: (i) construction of water-based dispersion pesticide nanoformulation, (ii) mechanism on leaf-targeted deposition and dose transfer of pesticide nanodelivery system, (iii) mechanism on increased bioavail-ability of nano-based pesticide formulation, and (iv) impacts of nanoformulation on natural degradation and biosafety of pesticide residues.

4 Construction of water-based dispersion pesticide nanoformulation

The fundamental limitation with the use of current pesticides is that they are generally comprised of virtually insoluble compounds (Stackelberg et al., 2001). This lack of solubility requires the addition of large amounts of organic solvents for dissolution and spraying application in the field, which increases costs, exposures of applicators, and environmental pollutants (Lawrence et al., 2006). Water-based dispersion pesticide nanoformulations improve the solubility and dispersion in water, uniform leaf coverage, biological efficacy, and environmental compatibility as a result of the small particle size, high surface area, and elimination of organic solvents in comparison to conventional formulations (Lawrence et al., 2006; Pratap et al., 2008; Anton et al., 2008).

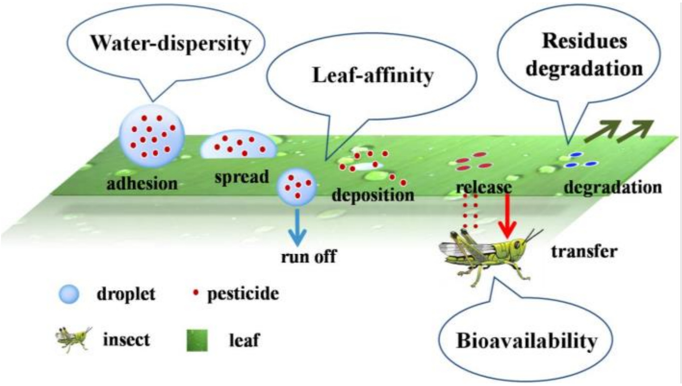

Figure 6. Four critical factors regarding the development of nanobased pesticide formulations

Synthesis of nano-based formulations involve size reduction by top-down methods, such as milling, high-pressure homogenization, and sonication, while bottom-up processes involve melt dispersion, solvent displacement, complex coacervation, interfacial polymerization, and emulsion diffusion (Nuruzzaman et al., 2016; Sasson et al., 2007). Nanocapsules, nanoemulsions, nanospheres, nanomicelles, and nanosuspensions show great potential for improving formulation properties, such as water dispersion, chemical stability, targeting adhesion, permeability, and controlled release (Figure 7).

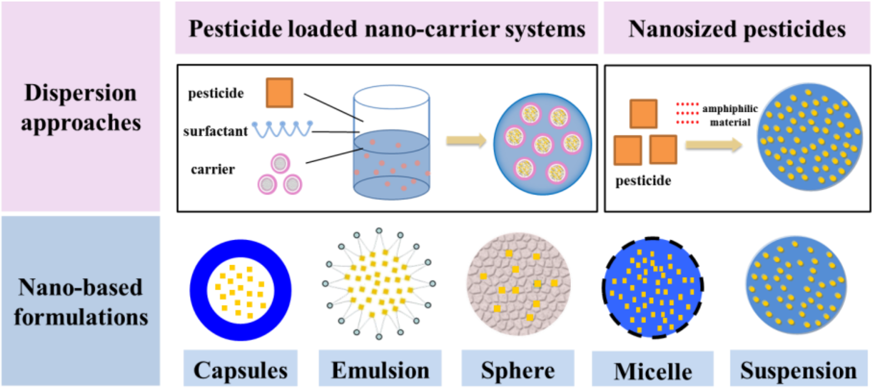

Figure 7. Schematic representation of water-based dispersion pesticide nanoformulation

Nanocapsules are core-shell structural vesicular systems, encapsulating the pesticide AIs in the inner core. The shell is usually composed of biodegradable polymers, including poly-ε- caprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), chitosan, etc (Cao et al., 2008; Wang et al., 2009; Xie et al., 2008; Sinha et al., 2004; Pereira et al., 2014; Campos et al., 2015; Wu et al., 2005; Li et al., 2011). The polymeric shell degrades slowly in the environment and, thus, improves the chemical stability for environmentally sensitive compounds [i.e., ultraviolet (UV) and soil degradation]. In addition, nanocapsules can increase the targeting delivery efficiency with membranal polymeric leaf-affinity modification, improving the behaviors of wetting, spreading, and absorbing of droplets on leaves.

Nanoemulsions are oil-in-water (O/W) emulsions, where the pesticides are dispersed as nanosized droplets in water and the surfactant molecules are localized at the pesticide-water interface (Mason et al., 2006; Koroleva et al., 2012; Wang et al., 2007). Nanoemulsions improve the efficacy and safety effects of traditional pesticides as a result of the small size effect, high dissolution rate, and elimination of toxic organic solvents. Nanospheres are solid sphere vesicular systems, where the pesticides are uniformly distributed through adsorption or entrapment inside the nanomatrix (Polshettiwar et al., 2010; He et al., 2015; Wu et al., 2013; Li et al., 2007). Nanospheres are composed of organic polymer materials or inorganic mesoporous materials, such as activated carbon, non-metal oxides, and porous hollow silica. Nanospheres possess high drugloading capacity, good biocompatibility, and slow/controlled release pattern, showing great potential in soil infection disease and soil pest control (Tang et al., 2012; Popat et al., 2012; Wanyika et al., 2013).

Nanomicelles are ideal, bioactive, smart, nanodelivery systems for encapsulating pesticides. Nanomicelles can be induced by the external environment and, thus, make the corresponding changes in physical and chemical properties. For example, on the basis of the hydrogen-bonding cross-linked nanomicelle, an environmentally responsive controlled release system was constructed. Under high-temperature and highhumidity conditions, the hydrogen bonding fractured, the nanomicelle swelled, and the pesticides were released. The pesticides were blocked under low-temperature and lowhumidity conditions the other way around (Li et al., 2009).

Naonosuspensions are pesticide nanoparticles uniformly suspended in water. The aqueous colloid dispersion systems render higher solubility and dispersion for insoluble or fatdispersible compounds in solution, improve the pesticide bioavailability, and reduce the costs as a result of the ease to large-scale manufacture.

5 Mechanism on leaf-targeted deposition and dose transfer of the pesticide nanodelivery system

The pesticide spray application on foliage is inadequate as a result of the weak adhesion to the crop foliage. For the spray pesticides, pesticides are first deposited on crop foliage and then they go to parts of the plant attacked by a pest through diffusion, uptake, and/or transfer processes, leading to pest poisoning or death by active or passive contact (Nuruzzaman et al., 2016; Yu et al., 2017). Consequently, leaf hydrophobicity and pesticide droplet retention are key parameters affecting the effective utilization of pesticides. As shown in Figure 8, the pesticide droplet forms a spherical shape, minimizing contact with the hydrophobic foliage, poorly wetting and spreading on the waxy layer, and resulting in loss with rolling down and runoff. After water evaporation, the residual pesticide particles easily drift or fall off the leaves because the particles are too large to embed in the micro- or nanostructured mastoids of leaf surfaces.

Nanodelivery systems form stable dispersions, increase the efficiency, and improve the wetting and spreading behavior on the leaf surface as a result of the leaf-affinity modification of pesticides. In addition, the pesticide nanoparticles deposit and adhere favorably on the surface of foliage, leading to an increased retention rate and a decreased spraying dosage (Figure 8). The adhesion properties of nano-based formulation were achieved by the multimodal interactions between the nanoparticles and the crop foliage, such as hydrogen bonding, electrostatic attraction, and covalent bonding (Jia et al., 2014). The adhesion strength strongly depended upon the size distribution of nanoparticles and the functional groups on the nanoparticle surface and was easily regulated by size controlling and varying functional groups (Yu et al., 2017). Carboxyl-modified nanocapsules reduced the surface tension of pesticide dispersions, decreased the contact angle of the droplet on hydrophobic foliage, and improved the retention rate (Liu et al., 2016; Li et al., 2016). Additionally, increased leaf coverage, improved diffusion properties, and penetration into plants were observed (Boehm et al., 2003; Cameron et al., 2007).

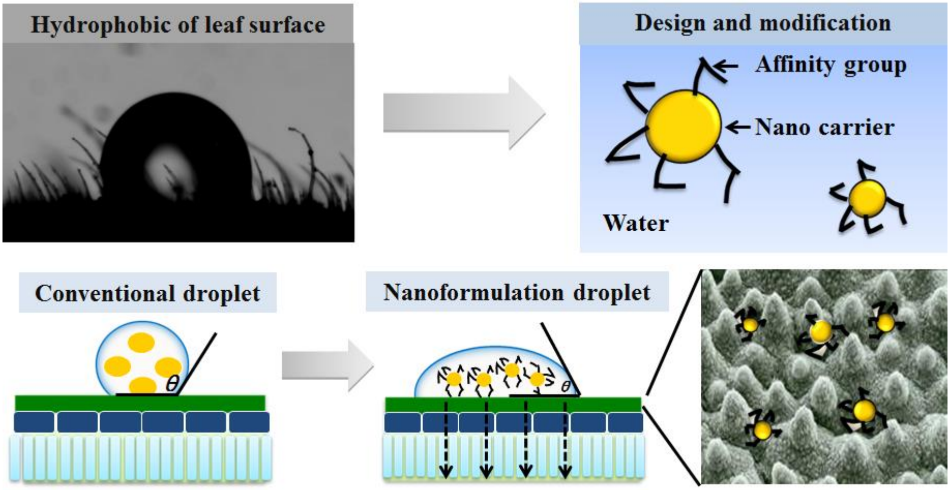

Figure 8. Pesticide deposition efficiency and dose transfer mechanism

6 Mechanism on the increased bioavailability of nano-based pesticide formulation

In comparison to the traditional pesticide formulation, nano-based formulations have a smaller particle size and larger specific surface area, which can effectively increase the coverage, adhesion, and permeability of the pest. In addition, nano-based formulations may affect the action modes and transfer paths of conventional pesticides by introducing insect target modification and enhancing the release of AIs (Figure 9). Pesticides can be classified according to four distinctive functions: stomach poisoning (the pesticide enters the body of pests via their mouthpart and digestive system), inhalation poisoning (the pesticide enters the body of pests via fluids from a consumed host organism), contact poisoning (the pesticide enters the body of pests via their epidermis upon contact), and fumigation (the pesticide in gas form enters the body of pests via their respiration system). It was presumed that nano-based formulation might enhance the stomach- and contact-poisoning functions, because it significantly improves the dispersal and permeability and, thus, increases the rate of the pesticide entering pest bodies (Lossbroek et al., 1988; Yang et al., 2017). Furthermore, the enhancement of the transport, conduction, and transformation efficiency of pesticide nanoparticles inside pests can accelerate pest poisoning, further improving the efficacy, bioactivity, and dose effect of pesticides (Liu et al., 2015).

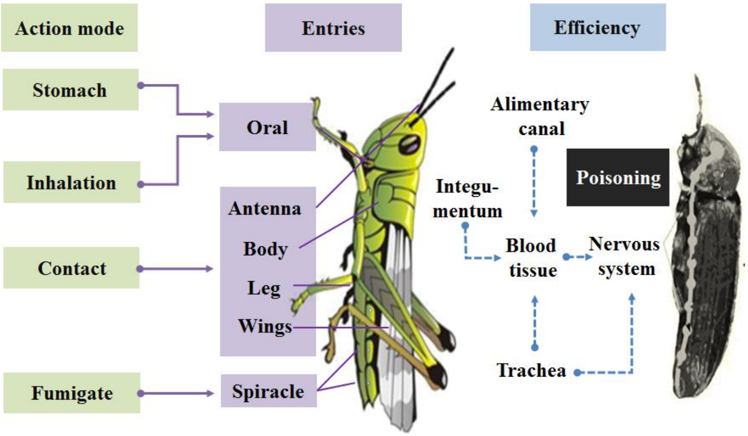

Figure 9. Bioavailability of the pesticide nanodelivery system

7 Impacts of nanoformulation on degradation and biosafety

Inevitably, nanoparticles will be released into the plants and environmental systems. The unique physical and chemical properties of nanoparticles might cause some unpredictable adverse effects on crops, agricultural products, and ecosystem (Service, 2003; Service, 2004; Brumfiel et al., 2003; Masciangioli et al., 2003;). In addition, these materials will accumulate over time in soils, and rates may vary in response to unknown parameters (Boxall et al., 2007; Gottschalk et al., 2009). The general concern is that some nanoparticles or nanostructured materials may flow into the environmental systems and food chain, which may become a new class of pollutant resources that threaten human health and ecosystem balance. However, because farmland is an open complicated system with many influencing factors of complicated functions, actual data measuring the environmental concentration of nanoformulations in various media is scarce (Bai et al., 2009; Mueller et al., 2008; Gottschalk et al., 2013). The environmental fate and potential biosafety problem of nanomaterials or nanoparticles from nanoformulations are also unclear (Klaine et al., 2008). Therefore, avoiding risk research should be conducted on safety and risk assessments of nanopesticides according to the methodologies established in nanotoxicology and nanomedicine. Investigating the toxicological effect, environmental behavior, and pharmacokinetics of nanoparticles, studying the interaction mechanism between nanoparticles and plants, and evaluating their potential impact on the quality and safety of agricultural products can provide a theoretical basis for the development of nanopesticides and the sustainable implementation of nanotechnology in agriculture (Figure 10). On the other hand, nano-based pesticide formulation can accelerate the catalytic degradation of toxic residues and reduce the pesticide residues in the environment by introducing biodegradable material carriers and photocatalysts (Caboni et al., 2003).

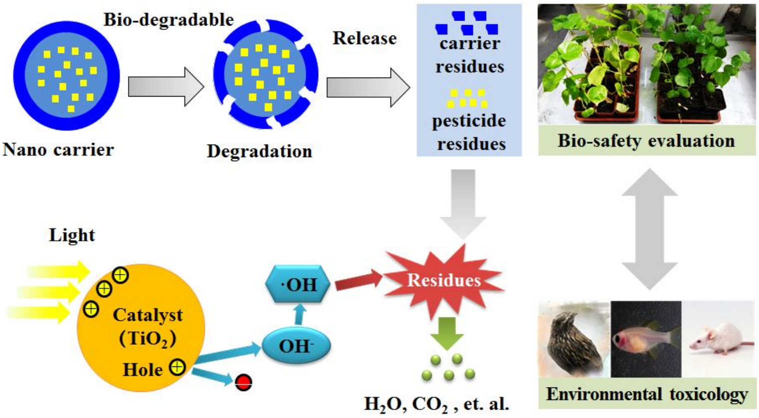

Figure 10. Catalytic degradation and biosafety of pesticide residues

8 Conclusion and prospects

Clearly, nano-based pesticide formulations have many advantages over the conventional equivalents, such as high efficiency, environmental friendliness, high targeting delivery, and smart controlled release. As a result of the technological advancement, large-scale applications of nanopesticides in crop production have just become possible. These are the desired properties and research objectives of nano-based pesticide formulations as summarized in Table 1.

Table 1. Desired Properties and Research Objectives of Nano-based Pesticide Formulations

Desirable Properties	Research objectives of nanopesticide-enabled technologies	Reference
Targeted delivery	increasing targeted delivery efficiency of the pesticide intoaction targets, such as plants, insects, and pathogen	Pandey et al., 2016; Hayles et al., 2017; Auffan et al., 2009; Kah et al., 2014.
Controlled release	controlling release of the pesticide at the least effective concentration for killing pests and pathogens	Kah et al., 2014; Ao et al., 2013; Liu et al., 2002; Boehm et al., 2003; Qian et al., 2011; Wang et al., 2012; Li et al., 2006; Sarkar et al., 2012; Pankaj et al., 2012.
Water dispersion	increasing solubility and dispersion for fat-soluble chemicals in aqueous solution	Frederiksen et al., 2003; Rabinow et al., 2004; Liu et al., 2011; Shang et al., 2006; Whitehouse et al., 2010; Pratap et al., 2008; Anjali et al., 2012; Mason et al., 2006; Koroleva et al., 2012
Chemical stability	improving chemical stability for light-sensitive compounds by restricting photodegradation	Liu et al., 2002; Polshettiwar et al., 2010; He et al., 2015; Wu et al., 2013; Li et al., 2007; Tang et al., 2012; Popat et al., 2012; Wanyika, 2013; Li et al., 2009; Yu et al., 2017; Jia et al., 2014; Cameron et al., 2007.
Bioavailability	increasing bioavailability for saving pesticides	Yang et al., 2017; Liu et al., 2015.
Lasting validity period	reducing the pesticide application and treatment frequency by extending the lasting validity period	Wanyika et al., 2013; Yu et al., 2017; Jia et al., 2014; Cameron et al., 2007.
Lower toxicity	protecting biodiversity in the ecosystem	Pandey et al., 2016; Hayles et al., 2017; FAO, 2010.
Environmental friendliness	reducing food residues and non-point source pollution as a result of the minimum pesticide loss	Kah et al., 2013; Kah et al., 2014; Guan et al., 2010; Matthews et al., 2000.

In conclusion, nano-based pesticide formulations bring beneficial improvements in properties and behaviors of traditional pesticides, such as solubility, dispersion, stability, targeting delivery, and controlled release of AIs. Additionally, it might not only significantly improve the bioavailability and duration of drug efficacy but also reduce the toxicity of nontarget wildlife, food, and environmental residues. On the other hand, some toxic nanoparticles from pesticides may flow into the environment and food systems and threaten human health and ecosystem balance. Avoiding risk research should be conducted on safety and risk assessments of nanopesticides according to the methodologies established in nanotoxicology and nanomedicine. Safer and biodegradable nanomaterials should be developed for nanopesticide production. As a most promising and attractive field of nanotechnology application in agriculture, these novel agrochemical products will provide multiple benefits, such as reduced use of chemicals and, subsequently, reduced water pollution and food product residual contamination, efficient use of agricultural resources, and increased soil and environmental qualities.

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The development of nanotechnology in pesticide delivery aims to reduce the indiscriminate use of conventional pesticides and ensure their safe application. This technology is relatively new and in the early stages of development. Among all the nano-based formulations, polymer-based formulations have received the greatest attention over the last two years, because they seem to have the greatest potential for further practical application. In this chapter, we discuss the development of nano-based polymeric insecticides, including the natural and synthetic carrier materials, the basic structures of nanoformulations and their efficacy. However, investigations into the environmental fate of nano-formulated insecticides remain scarce, and the current state of knowledge does not appear to be sufficient for a reliable risk assessment. So, many challenges, including reduction of the production cost and assessment of their performance, especially at field level, must be solved before their future marketing.

2.2 Polymer-Based Nanoinsecticide: Current Developments, Environmental Risks and Future Challenges

Abstract: The rapid development of the nanotechnology industry opens new perspectives for modern crop protection strategies. This review summarizes and discusses the use of polymers as carriers of nanoinsecticides. They are expected to ensure a higher level of protection for humans and the environment, while ensuring good efficacy of the active ingredient. Some of the synthetic polymers (including polyethylene glycol, polylactic acid, polycaprolactone and polyhydroxybutyrate) that are widely used in pharmaceutical or cosmetic areas can be employed as insecticide carriers. But natural polymers (including chitosan, alginate, cellulose, starch and cyclodextrins) are receiving increasing attention because of their environment-friendly properties. The polymeric materials can be prepared in various types of tridimensional structures, among which nanocapsule, nanosphere, micelle, nanogel and nanofiber are the most common for the delivery of the active ingredients. The environmental risks of polymer-based nanoinsecticides are highlighted, together with the main challenges that must be solved before their future marketing. These challenges include the reduction of their production cost and assessment of their performance, especially at field level.

Keywords: Polymer, nanoinsecticide, formulation, environmental risk

1 Introduction

Nanotechnology is considered as the fifth revolutionary technology of the last hundred years, after biotechnology (Chhipa, 2017a). Nanoscale materials are increasingly used in electronics, energy, medicine and life sciences (Nair et al., 2010), which benefit from their small size, chemical composition, surface structure, solubility, shape and aggregation (Nel et al., 2006). In recent two decades, the knowledge accumulated in these areas is being transferred and adopted in the agricultural sector, facilitating the development of plant protecting agrochemicals (Mattos et al., 2017). In order to avoid the deleterious effects of pesticides, the agrochemical industry looks for new active ingredients (AI), but also develops new pesticide formulations (Villaverde et al., 2017), helped by the development of nanotechnology.

The key motivation to develop nanoformulations is to improve the efficacy of pesticides, while lowering doses and application frequency. Indeed, nanoformulations should allow regular, precise, long and targeted delivery (Khandelwal et al., 2016), which also reduces environmental contamination and exposure to human and other non-target organisms (Pascoli et al., 2018). Ideally, a pesticide should maintain an adequate AI level for pest control and leave minimum residue in crops and in the environment. This can be achieved by encapsulating pesticide in polymeric controlled release systems, where the polymer properties can be adapted by modifying the molecular weight and basic structure of the polymer, according to actual needs (Roy et al., 2014). This is the reason why most of the recent literatures make the controlled release of AI as the primary objective of polymer-based nanoformulations (Kah et al., 2013).

According to the meta-analysis of Kah et al (2014) performed on nanopesticide publications (2000-2013), insecticides accounted for 55% of the peer-reviewed publications. This high proportion can be explained by the facts that AI of many conventional insecticides have poor water solubility, are sensitive to the environmental factors, and easy to volatize or degrade. In order to decrease the amount of organic solvent put in the environment and prevent the premature degradation of AI, a delivery system is required for the application of insecticides. Polymer-based nanoformulations are suitable for a great number of applications, including slow release of AI, protection against degradation and increased solubility of AI.

In this review, we focus on the development of the polymer-based nanoinsecticides, including the polymeric materials, the AI formulations and their efficacy. In addition, our attempt was to describe the environmental risks and future challenges of polymer-based nanoinsecticides, which have received a great deal of attention in recent years.

2 Current Development of Polymer-based Nanoinsecticides

Polymeric nanoparticles are among the most important nanostructured systems used for controlled release of drug formulations. They were recently employed for pesticide delivery (Nuruzzaman et al., 2016). In general, AIs are loaded or entrapped with polymers, which are within the nano-range of 1-1000 nm, at least for one of their dimensions (Kah et al., 2013; Nuruzzaman et al., 2016). Environment-friendly polymers are more suitable as carrier materials for AI. The most popular shapes of polymer-based nanoinsecticides are nanoencapsule, nanosphere, micelle, nanogel and electrospun nanofibers (Perlatti et al., 2013; Kah et al., 2014).

2.1 Polymeric materials

A large group of nanoinsecticide-focused research papers explores the applicability of new polymeric materials for plant protection (Kah et al., 2014). With an increasing awareness of environmental protection, more and more polymers of natural or synthetic origins are used as nanoinsecticide carriers. The qualities of these polymeric materials typically include the fact that they are mostly easy to degrade, leaving no secondary pollution and are available at low-cost (Perlatti et al., 2013). Various polymer-based nanoinsecticides and their efficacies are listed in Table 2.

2.1.1 Natural polymers

Natural materials are receiving increasing attention by the manufacturers, for all the reasons described above, but also because petroleum resources are diminishing all over the world. Natural polymeric materials and their derivatives are sustainable sources which are readily available, facilitating their large-scale production. The main natural polymers employed as carriers of nanoinsecticides are described below.

Chitosan is industrially produced by partial deacetylation of chitin, which is the primary component of the invertebrates' exoskeleton and of the cell walls of some bacteria and fungi (Campos et al., 2014). Because it is non-toxic, biodegradable and biocompatible, chitosan is regarded as one of the most promising polymeric materials for the efficient delivery of agrochemicals (Kashya et al., 2015), especially to build up nanoinsecticides. In the recent decade, research on the preparation of nanogels containing insecticidal essential oils (EO) using chitosan as carrier has become a hot spot (Abreu et al., 2012; Almeida et al., 2018; Ziaee et al., 2014a; Ziaee et al., 2014b). Also, due to the functional groups of the polymer chains, it is possible to make some structural modifications and obtain materials with improved properties. Xiang et al. (2017) developed a multifunctional nanopesticide system by coating collectable magnetic diatomite with chitosan, and the pH-responsively system loaded with cypermethrin showed a high adhesion capacity on pests' epidermis, resulting in an improved efficiency against corn borers under lab condition. Sun et al. (2014) encapsulated hydrophilic methomyl in shell cross-linked nanocapsules formed by the self-assembly of photocrosslinkable carboxymethyl chitosan, and the insecticidal activity test in laboratory against armyworm larvae was significantly better than the technical products.

Alginate is typically obtained from brown macroalgae and conventionally applied in food industry as emulsion stabilizers, gelling agents, film-forming agents etc.

Alginate polysaccharides are classified as hemocompatible materials and do not accumulate in any organs of the human body (Jerobin et al., 2012). They have been developed as carriers of nanoinsecticides via an ionotropic gelification process triggered by metal ions (Campos et al., 2014). Saini et al. (2014) prepared pyridalyl-loaded sodium alginate nanocapsules, and compared them with technical material and conventional formulation by leaf dip method, concluding that the nanoformulation showed better toxicity to shoot borer (*Helicoverpa armigera*). Kumar et al. (2014) produced imidacloprid-loaded sodium alginate nanoparticles, and carried out field efficacy assays on leafhopper of okra. Compared to the commercial formulation, the nanoparticles showed improved efficacy, control measure and long-lasting properties. In addition, the cytotoxicity of nanoparticles to Vero cells was lower than conventional formulation. However, it was found that nanocapsules formed only by alginate polymer might have low stability which resulted in loss of encapsulated AI (Kumar et al., 2015). So, sodium alginate is used together with other polymeric materials such as chitosan, starch and poly (ethylene glycol) for overcoming the limitations associated with swift release of AIs (Jerobin et al., 2012; Kumar et al., 2015).

Cellulose is the most abundant natural polymer in nature. Because of its useful properties including biodegradability, biocompatibility, low toxicity and low-cost, cellulose and its derivatives are intensively used as delivery system for medical therapy (Gopinath et al., 2018). Since they can be degraded by many naturally-occurring bacteria and fungi, these polymers are gradually employed as carriers of agricultural compounds. However, there are only a small portion of research papers focusing on the insecticidal efficacy of nanoinsecticides based on cellulose (and derivatives). Shoaib et al. (2018) synthesized emamectin benzoate (EB) loaded ethyl cellulose nanocapsules, and tested the insecticidal activity of nanocapsules against *Plutella xylostella* by leaf dipping method. They however found no significant difference between ethyl cellulose nanocapsules and technical grade EB, whereas the nanocapsules could effectively protect EB from photolysis. Zhao et al. (2013) prepared ultrafine fiber of cellulose acetate that contained avermectin via an electrospinning process, resulting in a continuous release to fulfill the effective utilization of avermectin.

Starch is the energy storage molecule of most green plants and is found in grains, roots, legumes and fruits. This polymeric hydrocarbon is made of a large number of glucose units and is easily available at low-cost. Starch and starch-based materials have showed great potential for food, medical and agricultural applications. However, the poor water solubility and processability of native starch makes it difficult to process under mild conditions. Physical or chemical modifications have been adopted to improve its properties and adequacy as nanoinsecticide formulation: Li et al. (2016) prepared avermectin loaded starch capsules with a diameter range of 0.7-4.8μm by prexim membrane emulsification method. The capsules with avermectin contents of 16-47% enabled a controlled and consistent release of the insecticide over a two weeks period. Ihegwuagu et al. (2016) assessed that the addition of nanosilver into cassava starch improved the encapsulation efficiency of dichlorvos and chlorpyrifos to 95-98%, which was attributed to the enhanced surface area of the nanoparticle. Moreover, silver nanodichlorvos and nanochlorpyrifos insecticides could achieve highly effective and sustained release lasting for 21 days.

Cyclodextrins are the products of enzymatic degradation of starch, and consist of a macrocyclic ring of six, seven or eight glucose subunits (α, β, and γ cyclodextrins, respectively) (Campos et al., 2015). Cyclodextrins have a truncated cone structure, which contain a hydrophobic inner cavity and a hydrophilic, polar outer surface (Campos et al., 2015). Such a conformation enables cyclodextrins to form non-covalent inclusion complexes with various hydrophobic molecules, and impacting the biological, chemical and physical properties of the included molecule (Yusoff et al., 2016). Petrović et al. (Petrović et al., 2011) modified β-cyclodextrins with methyl epoxycinnamate to boost the solubility of organic pesticides, and the modified β-cyclodextrins had a significantly better solubilizing effect on dimethoate than on other pesticides. Carvalho et al. (2012) studied the efficacy of six neem oil nanoformulations encapsulated in β-cyclodextrins and polycaprolactone against eggs and nymphs of *Bemisia tabaci* Gennadius. However, none of these six nanoformulations provided better efficacy results than the commercial neem oil, which might be caused by the slow rupture of the polymer and the gradual release of AI.

2.1.2 Synthetic polymers

One of the common objectives of developing polymeric nanoinsecticides is to produce less harmful plant-protection products, similar to the biodegradable synthetic polymers used in the pharmaceutical or cosmetic areas. Usually, these synthetic polymeric materials are nontoxic, or can be degraded by microbes, and their decomposition products are eco-friendly. The most common synthetic polymers used as carriers of nanoinsecticides are described below.

Polyethylene glycol (PEG) is linear or branched, neutral polyether of a variety of molecular weights, and soluble in water and most organic solvents. Because of its wide range of solubility and safety properties such as lack of toxicity, no antigenicity and immunotoxicity, non-interference with conformations of polypeptides and enzymatic activities and ease of excretion from living organisms (Danprasert et al., 2003), PEG has been approved by the USFDA (D'souza et al., 2016) and widely used in drugs. PEG-based nanoformulations have great potential in pest control. Balaji et al. (2015) formulated nanomicelles of a poor water-soluble insect repellent, diethylphenylacetamide, by PEG polymerization followed by phase inversion temperature emulsification, and the nanoformulated diethylphenylacetamide exerted better bioefficacy against Japanese encephalitis vector *Culex tritaeniorhynchus*, in comparison with its bulk form, even at minimal exposure concentrations. Werdin González et al. have intensively studied the efficacy of PEG-based EO nanoparticles on mosquitoes *Culex pipiens pipiens* (Werdin González et al., 2017), german cockroaches *Blattella germanica* (L.) (Werdin González et al., 2016; Werdin González et al., 2015), stored product beetles *Tribolium castaneum* and *Rhizopertha* *dominica* (Werdin González et al., 2014), and these results all showed that EO nanoparticles led to higher efficacy than EO alone.

Polylactic acid (PLA) is also a USFDA-approved polymeric material which is widely used as drug or cell carrier in the medical field for its biodegradable and mechanical properties that can be adjustable (Lee et al., 2016). PLA degrades into lactic acid, and its final metabolized products *in vivo* are carbon dioxide and water. In recent years, research on PLA-based nanoinsecticides has gradually increased. Liu et al. (2016) fabricated controlled delivery system for Lambda-Cyhalothrin (LC) with PLA as the carriers, through premix membrane emulsification. They found that microcapsules with tunable sizes ranging from 0.68 to 4.6 µm had better water dispersion and longer rates of release. The 0.68 µm LC-loaded microcapsules exhibited a similar biocidal efficacy against *Plutella xylostella* as that of a commercial microcapsule formulation. Yu et al. (2017) developed three types of functionalized abamectin PLA nanoparticles with different adhesive abilities to cucumber leaves. They found no difference between adhesive PLA nanoparticles, commercial water dispersible granules, and emulsifiable concentrate, in a bioassay performed on cucumber aphids.

Polycaprolactone (PCL) is a biodegradable polyester, intensively used as controlled release drug carrier and tissue engineering due to its biocompatibility and miscibility with a large range of other polymers (Dash et al., 2012). Khoobdel et al. (2017) prepared *Rosmarinus officinalis* EO loaded PCL nanocapsules, which had a higher toxicity against red flour beetle (*Tribolium castaneum)* than the non-formulated EO. Zhang et al. (2017) used an amphiphilic block copolymer, polyethylene oxide-b-poly(caprolactone) (PEO–PCL), to make ricinine nanomicelles, which were easy to wash-off from the trial leaves and meanwhile enhanced the protection against *Tetranychus cinnabarinus (B.)* during field trials.

Polyhydroxybutyrate (PHB) is a biocompatible and biodegradable material that can be obtained from renewable sources, though the cost is higher than other synthetic polymers (Dos Santos, et al., 2017). Publications related to PHB nanoinsecticide formulations are scarce. Giongo et al. (2016) developed nanoformulations of neem (*Azadirachta indica* A. Juss.) including colloidal suspension and powder containing PHB, in capsules or spheres. Bioassay results against fall armyworm larvae showed that PHB neither caused adverse effects on insects, nor interfered with the action of neem. In comparison to commercial neem oil, PHB nanoformulations were as efficient in reducing larval weight, though the mortality was relatively low.

Because of the modification and miscibility of these materials, carriers based on polymers, such as copolymers, inorganic carriers mixed polymers and surface modified functionalized polymers are developed. In one word, polymer-based nanoformulations have a great potential for further development and practical crop protection applications (Kah et al., 2014).

Table 2. List of Polymer-Based Nanoinsecticides

Nanostructure	Polymers	Active ingredients	Targets	Efficacy of Nanoformulations	Reference
Nanocapsule	Poly (citric acid) (PCA) and PEG copolymers	Imidacloprid	Larvae of *Glyphodes pyloalis*	LC_{50} decreased over free imidacloprid	Memarizadeh et al, 2014
	Azidobenzaldehyde (Az) and carboxymethyl chitosan (CMCS)	Methomyl	Armyworm larvae	Significantly superior to the technical product	Sun et al, 2014
	Ethyl cellulose	Emamectin benzoate	*Plutella xylostella*	No significant difference between the nanocapsules and the technical product	Shoaib et al, 2018
	PEG	Diethylphenylacetamide	*Culex quinquefasciatus*	Improved efficacy to the bulk even at lower concentrations	Balaji et al, 2017
	PEG	Acephate	*Spodoptera litura* and *Oligonychus coffeae*	Significantly more efficient than commercial formulation	Pradhan et al, 2013
	PLA	Lambda–Cyhalothrin	*Plutella xylostella*	Similar efficacy as a commercial formulation	Liu et al, 2016
	PCL, PHB, poly (methyl methacrylate) (PMMA)	Neem extraction and oil (*Azadirachta indica A. Juss.*)	Fall armyworm	Lower than commercial neem oil	Giongo et al, 2016

	PCL	*Rosmarinus officinalis* EO	*Tribolium castaneum*	Higher toxicity than non-formulated EO	Khoobdel et al, 2017
	Sodium alginate	Pyridalyl	Larvae of *Helicoverpa armigera*	More effective than technical material and commercial product	Saini et al, 2014
	PCL	Neem (*Azadirachta indica*) oil	*Bemisia tabaci* Biotype B	Less efficient than the commercial neem oil	Carvalho et al, 2012
Nanosphere	Chitosan and sodium tripolyphosphate	Nicotine hydrochloride	*Musca domestica*	Shorter KT_{50} , higher 24 h mortality than suspo-emulsion, and effective duration of more than 30 days	Yang et al, 2018
	Chitosan modified magnetic diatomite	Cypermethrin	Corn borers	Slow release property controlled by pH of solvent, almost same efficacy with commercial Cyp and Cyp	Xiang et al, 2017
	PEG and chitosan	*Geranium maculatum (L.)* and *Citrus bergamia* (Risso) EOs	*Culex pipiens*	Chitosan nanoparticles produced higher acute and residual activity than PEG nanoparticles and EO alone	Werdin González et al, 2017
	PEG	*Geranium maculatum (L.)* and *Citrus bergamia* (Risso) EOs	*Blatella germanica*	Higher efficacy than EO alone, also exerted sublethal effects	Werdin Gonzalez et al, 2016
	PEG	*Geranium sp.* and *Citrus reticulata L.* EOs	*Blatella germanica*	Increased residual contact toxicity and contact toxicity than EO alone	Werdin González et al, 2015
	PEG	Geranium and bergamot commercial EO	*Tribolium castaneum* and *Rhizopertha dominica*	Enhanced the EO contact toxicity and altered the nutritional physiology of both pests.	Werdin González et al, 2014

	PEG	Citrus peel EO	*Tuta absoluta*	Higher mortality on adults than EO alone	Campolo et al, 2017
	PEG	Fipronil	*Nilaparvata lugens*	Higher mortality than commercial formulation in field test	Kumar et al, 2018
	PEG	β-cyfluthrin	*Callosobruchus maculatus*	More efficacious than commercial variety	Loha et al, 2012
	Sodium alginate	Imidacloprid	Jassids/leafhoppers	More superior effect in efficacy, control measure and long lasting	Kumar et al. 2014
Nanogel	Chitosan and cashew gum	*Lippia sidoides* EO	Third instar *St. Aegypti* larvae	More effective larvicide efficacies than the pure EO.	Abreu et al, 2012
	Myristic acid-chitosan	*Cuminum cyminum L.* EO	*Sitophilus granarius L.* and *Tribolium confusum*	More toxic than EO alone, improved the persistence of the encapsulated EO	Ziaee et al, 2014
	Myristic acid-chitosan	*Carum copticum (L.)* EO	*Sitophilus granarius L.* and *Tribolium confusum*	8.9- and 3.7-fold more toxic than the EO against *S. granarius* and *T. confusum*	Ziaee et al, 2014
	Isocyanate-terminated star-shaped poly(ethylene oxide-stat-propylene oxide) and β-Cyclodextrin	Permethrin	Larvae of *Tineola bisselliella* and of *Anthrenocerus australis*	Good protection as commercial formulations even in low AI concentrations	Kettel et al, 2014

	Low-molecular mass gelators (LMMGs) all-trans tri(p-phenylenevinylene) bis-aldoxime	Pheromone methyl eugenol	*Bactrocera dorsalis*	More effective than ME alone, and remained active over a sustained period in field trial.	Bhagat et al, 2013
Micelle	PEG	Diethylphenylacetamide (DEPA)	*Culex tritaeniorhynchus*	Better bioefficacy as comparative to its bulk form even at minimal exposure concentrations	Balaji et al, 2015
	Poly(ethylene oxide)-b-poly(caprolactone) (PEO–PCL)	Ricinine	*Tetranychus cinnabarinus (B.)*	Better acaricidal efficiency than the Tween-80 formulations	Zhang et al, 2017
Electrospun nanofiber	Polyamide 6 Cellulose acetate	Pheromone (Z)-9-dodecenyl acetate	--	An almost linear release over several weeks	Hellman et al, 2011
	PLA/cellulose nanocrystal	Thiamethoxam	Whiteflies	Efficient at 50% of the recommended dosage over 9 days	Xiang et al, 2013

2.2 Structures of polymer-based nanoinsecticides

The polymeric materials can be prepared in various types of tridimensional structures, among which nanocapsules, nanosphere, micelle, nanogel and nanofiber are the most common for AI delivery (Figure 11).

Nanocapsule: the AI is concentrated near the solid or liquid inner core that is lined by the protective shell of polymeric materials. Ojha et al. (2018) and Nuruzzaman et al. (2016) have generalized the methods to produce nanocapsules. Owing to the homogeneous distribution, nanocapusules may be more stable for spraying, increase the utilization rate of AI and reduce the phytotoxicity. Controlled release studies indicated that nanocapsules exhibited a higher release rate than the microcapsules because specimen with smaller size possesses larger surface areas being exposed to the surroundings (Li et al., 2016; Liu et al., 2016). However, it is still a challenge to design nanoscale capsules with high AI loading rate.

Nanosphere: the AI is uniformly distributed and embedded in the polymeric matrix. If the distribution of AI within a so-called capsules or core/shell nanoparticles is uncertain, these formulations should be considered as nanospheres (Kah et al., 2013). Although the synthesis process of nanospheres is very similar to that of nanocapsules, the technique of polymerization is still very important. The size, dispersity, and loading efficiency of the nanospheres always change when a different type of surfactants is used (Ojha et al., 2018). Nanospheres can serve as protective reservoirs and controlled release carriers, which bring about a longer protection and a reduction of leaching losses (Kah et al., 2013). The small size of the nanospheres could also enhance the penetration of AI in the plants, and consequently improve the efficiency of the AI (Boehm et al., 2003).

Micelle: core-shell structured micellar systems are self-aggregated in aqueous solutions by copolymers containing hydrophilic and hydrophobic moieties above the critical micellar concentration (CMC). Because of the large amount of interaction points of polymer chains, polymeric micelles show lower CMC values than surfactant micelles, which indicate better thermodynamic stability (Chen et al., 2014). Micelles are mainly used to deliver water-insoluble agrochemicals (Balaji et al., 2014; Zhang et al., 2017).

Nanogel: hydrophilic polymers cross-linked by van der Waal’s forces or covalent bonds, which can absorb high volumes of water. Nanogels are not likely to swell or shrink with changes in humidity due to the insoluble properties and they can improve the loading and release profiles of AIs (Kah et al., 2014). They have been intensively studied as the carrier of pheromones and Eos (Abreu et al., 2012; Ziaee et al., 2014a; Ziaee et al., 2014b; Kettel et al., 2014; Bhagat et al., 2013).

Electrospun nanofiber: polymer injection produced by the metal capillary forms nanofibers under the action of an electric field and collected by a collector (Noruzi, 2016). Though still in the early stage of agricultural application, electrospun nanofibers own potential advantages on avoiding the release bursts, which facilitates the field application of pheromones and EOs.

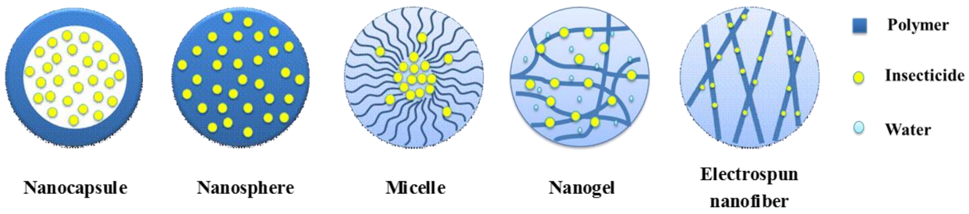

Figure 11. Different morphological forms of polymeric nanopesticides

3 Environmental Risks of Polymer-based Nanoinsecticides

Though nanopesticides may offer a range of benefits, they are still in the early developmental stage. Several companies have deposited patents comprising numerous protocols for production and application of nanopesticides, whereas only a small portion of nano-products using already registered AI have been marketed (Peters et al., 2016). During the last two decades, a great number of articles that related to the environmental health and safety of engineered nanoparticle have been published. However, research on evaluation of environmental safety of polymer-based nanopesticides is scarce, which results from the lack of standard approaches to assess the environmental risk of nanopesticides for regulatory purposes.

It is usually assumed that ecotoxicity of conventional pesticide is related to AI mass concentration. Environmental fate studies are usually undertaken only with AI or a representative formulation, all of whose ingredients have been approved (Amenta et al., 2015). However, as to nanopesticides, other parameters such as particle number concentration, particle size distribution (PSD), and the ratio of “free” and nanoparticle-bound AI, may be important in evaluating bioavailability and toxicity of pesticide (Kookana et al., 2014). Besides, it may also be very important to characterize these parameters at different stages in the environmental life cycle and throughout fate and effect studies. Nanopesticides will often undergo changes in their degree of dispersion or agglomeration over time, which depend on the concentration of the nanopesticides and environmental factors (Kookana et al., 2014). That is to say nanopesticides containing approved AI could be considered as a different pesticidal product, which would require a separate risk assessment and authorization.

Meredith et al. (2016) determined how the capsule size of one commercial lambda-cyhalothrin (λ-Cy) capsule suspension influenced on toxicity to embryonic zebrafish, *Danio rerio,* and the results showed capsule size did not influence the occurrence of sublethal impacts or mortality, but the presence of the capsules influenced the toxic response of the entrapped λ-Cy.

Polymer-based nanopesticides could reduce AI concentration, which may result in better environmental safety properties owing to lower environmental exposure and residues. On the other hand, however, slow release of AI may imply longer duration and consequently higher risk for non-target organisms and potentially greater amount of residues on harvest (Alia et al., 2016). De Oliveira et al. (2014) suggested that the effects of nanocarriers on soil microorganisms, pollinators, beneficial insects and other non-targeted organisms, together with the uptake and accumulation of nanoparticles in crop plants and their translocation to edible plant parts, should be studied in detail. Iavicoli et al. (2017) also considered the hazard identification of nanoformulation needs to focus on the AI concentration properties and the nano-component, but if the nano-component simply protects AI from degradation, the fate and behavior of the nano-component may be the same as in conventional pesticide formulation.

However, only one research was found on the biosafety evaluation of nanocarriers of insecticides, on different targets (Xiang et al., 2017): weed (*Cynodon dacylon*), pest (cotton bollworms), cell (HaCaT cells), bacteria (*Escherichia coli*), concluding that the nanocarrier possessesd a high biosafety with all concentrations. But the biosafety evaluation of nanoformulation on these targets was not elucidated. Some research focused on cytotoxicity of different cells, such as Vero cell line (Kumar et al., 2014), lymphocyte cells (Jerobin et al., 2012), MRC5 cell line (Pradhan et al., 2013), which is the common method for evaluating the biosafety of nanomedicine. Murine model was also adopted to evaluate the acute oral toxicity[8]. Pasquoto-Stigliani et al. (2017) not only assessed the cytotoxicity of neem oil loaded PCL nanocapsules on different cell lines, but also performed molecular analysis of the soil nitrogen cycle microbiota after treatment with nanocapsules, concluding that nanocapsules did not affect the soil microbiota during 300 days of exposure. Saini et al. (2015a; 2015b) evaluated the residue, dissipation and safety of sodium alginate-based pyridalyl nanoformulation on both tomato and okra, and results indicated that residues of nanopyridalyl did not persist much longer than that of conventional formulation, which implied the negligible risk to the humans.

Though guiding principles for evaluating environmental risks of nanopesticides has been suggested (Kookana et al., 2014), there is still no comprehensive study currently in the literature that evaluates environmental impact of nanopesticides under field conditions (Kah et al., 2018a). Since direct measurement are not easy to perform, indirect approaches, such as sorption and degradation that can be carried out in laboratory, are worth for assessing the fate and behavior of a nanopesticide. Kah et al. (2018b) compared the sorption and degradation of three polymer-based clothianidin (type of polymer not mentioned) formulations with pure AI and a commercial formulation, results showed that compared with conventional formulation, polymer-based nanoformulations increased the photodegradation half-life in water by a maximum of 21%; sorption to soil was increased by up to 51% and 10%, relative to pure clothianidin and the commercial formulation, respectively. Similar research work was carried out on bifenthrin in different type of soils by Kah et al. (2016); while sorption of commercial formulation was similar to those of the pure AI, significant differences were observed for the nanoformulations relative to the pure AI, which depended on the type of soil and type of formulations; and nanoformulation could prolong the persistence of bifenthrin as well. With the data obtained in the soil degradation experiment, soil persistence concentration and ground water concentration of nanopesticide could be predicted by simulating FOCUS models (1997, 2012).

Usually, the evaluation of environmental behavior is carried out after nanoformulation is prepared. Conversely, Petosa et al. (2017) first investigated the transport potential of four types of hollow polymeric nanocapsules in model soil systems, then one nanocapsule with moderate transport potential was chosen for loading bifenthrin. The impact of cation species, sand type, and ammonium polyphosphate fertilizer on the transport potential of nanoformulation was examined and compared to a commercial bifenthrin formulation, concluding that the nanocapsule was a promising delivery matrix.

4 Future Challenges of Polymer-based Nanoinsecticides

Although polymer-based nanoinsecticides are at an early stage of development, it is still expected that this technology will improve the efficiency of pesticide and reduce the environmental pollution. Therefore, more studies are required to solve the challenges faced by polymeric nanoformulations.

The main challenge associated with polymeric nanopesticides is to demonstrate that they could compete with existing formulations in both cost and performance, especially at field level. At present, nearly all the polymeric nanocarriers listed in literatures are synthesized in laboratory in very small amount, so it is necessary to establish common procedures for a particular group of pesticide, which could be scaled up for commercial level (Chhipa, 2017b; Nuruzzaman et al., 2016).

New analytical approaches are needed to fill the knowledge gap of the characterization of nanopesticides. Characterization data are extremely important to connect the novel qualities of the products with their physicochemical properties, to understand the relevant mechanisms, and to evaluate if the benefits are able to be preserved across a range of agronomic conditions (Kah et al., 2018a). Consisting of organic ingredient, the various forms of polymeric nanopesticides usually make their characterization difficult.

New experimental protocols for detecting and quantifying nanopesticides are necessary to understand their fate and to carry out the environmental impact assessment. At present, it is impossible to detect or quantify polymer nanocarriers in the soil matrix, because of the similarity of the elemental composition. Also, release rates of nanopesticides are most often measured in the laboratory with a dialysis method that is at considerable high concentration levels, and over relatively short periods of time, which is far from the real scene of pesticide application. Besides, modeling tools are also required to predict the transportation and relocation of nanopesticides.

Improvement in regulation for nanopeisticides is required urgently. EU (along with Switzerland) is the only world region where nano-specific materials have been incorporated in legislation, including specific information requirements for risk assessment of nanomaterials, and the obligation to label or report the presence of nanomaterials in products, but there is still no nano-specific guidance available for the risk assessment of nanopesticides (Amenta et al., 2015). Before new tools and techniques become available, it is necessary to identify if a new product could be treated as conventional pesticides, because some of the risk assessment approach, with proper modifications and adaptations, remains useful in several circumstances (Kookana et al., 2014).

5 Conclusion

Nanotechnology is one of the strategies that aim to maximize crop yields and minimize the input of pesticides. Polymer-based nanoformulations have received great attention recently because they appear to be promising for target release of AI while reducing excess run-off. However, it is still notable that the uptake, bioavailability and toxicity of nanoformulations are quite different from the conventional pesticides. Therefore, development of new methodologies is needed to understand the process. The environmental behavior and effects may also differ with their conventional analogues, and refined approaches for risk assessment are needed. In order to ensure a high level of protection for humans and the environment, while not hindering the development of new beneficial products, collaboration among countries around the world is required.

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3

Development of nanopesticides

From Sun, C., Wang, Y., Zhao, X., Zeng, Z., Cui, B., Shen, Y., Gao, F., Cui, H., 2018. Properties of avermectin delivery system using surfactant-modified mesoporous activated carbon as a carrier. J. Nanomater. 2018, 3038902.
From Yu, M., Sun, C., Xue, Y., Liu, C., Qiu, D., Cui, B., Zhang, D., Cui, H., Zeng, Z., 2019. Tannic acid-based nanopesticides coating with highly improved foliage adhesion to enhance foliar retention. RSC Adv. 9, 27096.

Avermectin is one of the most used pesticide around the world. However, its poor water-solubility and sensitivity to light may result in low pesticidal activity. Carbon materials, such as nanotube, graphene oxide, are often used as the nanocarriers of pesticides, because they can protect the active ingredients from degradation, and allows for sustained release of the active ingredients. However, the high cost of these materials makes it unpractical to market. So, in this chapter, low-cost mesoporous activated carbon was used as the nanocarrier of avermectin to improve the photostability and allow for sustained release. The loading capability of mesoporous activated carbon is mainly related to the pore structures and surface chemical properties. In order to further improve the loading rate of avermectin, two surfactants, sodium dodecyl sulfate and tetrabutylammonium bromide were employed to modify the carrier. The performance of surfactant-modified mesoporous activated carbon was characterized.

3.1 Properties of Avermectin Delivery System Using Surfactant-Modified Mesoporous Activated Carbon as a Carrier

Abstract-The sensitivity of avermectin to several environmental factors, especially light, causes low pesticidal activity and environmental pollution. In this study, surfactant-modified mesoporous activated carbon (MAC) was employed to absorb avermectin (Av) in order to improve its photostability and allow for sustained release of avermectin. The results suggest that sodium dodecyl sulfate (SDS)-modified MAC has excellent absorption of avermectin, and the absorption can be represented by the Langmuir isotherm model. The Av-MAC-SDS delivery system significantly improves sustained release of avermectin and also effectively inhibits the photodegradation of avermectin. These results indicate that SDS-modified MAC can be used as a carrier for avermectin to improve its pesticidal activity and reduce pesticide residues.

Key words: mesoporous activated carbon; avermectin; delivery system; sustained release; photodegradation

1 Introduction

Pesticides are indispensable in agricultural production. Environmental pollution caused by the misuse of chemical pesticides, however, is becoming more and more serious (Morillo et al., 2017; Rasmussen et al., 2015). Because of this, biopesticides have attracted increasing attention for their high bio-efficiency, safety, and other environmentally-friendly traits that are consistent with the requirements of sustainable agriculture (Mnif et al., 2015). Avermectin is a class of macrocyclic lactones isolated from the soil organism *Streptomyces avermitilis*. It has excellent pesticidal activity in agricultural systems due to high efficiency, low toxicity and high selectivity. However, its conventional formulations still have some shortcomings, such as environmental sensitivity and short duration of effect. In order to improve the pesticidal activity of avermectin, it is preferable to adsorb avermectin onto some forms of an adsorbent that can prevent degradation and consequently avoid the loss of pesticidal activity.

Activated carbon is an adsorbent material with a large surface area. Because of its well-developed pore structure and chemical stability, it has been widely used for purification, especially for purifying air and water (Suha et al., 2016; Korotta-Gamage et al., 2017). In the last decade, it has been extensively used for the prevention of environmental pollution (Bazan-Wozniak et al., 2017; Derylo-Marczewska et al., 2017; Macías-García et al., 2017), and in pharmaceutical applications (Miriyala et al., 2017) and the catalytic industry (Athappan et al., 2015) as well. As a carrier of chemical pesticides, activated carbon protects the active ingredients, and allows for and sustained release of the active ingredients (Sjogren et al., 1996). However, research about mesoporous activated carbon (MAC) loaded with biopesticides has been limited. Our previous work shows that MAC allowed for sustained-release and UV-shielding of avermectin (Sun et al., 2010), and the surface acidic groups of MAC, especially carboxyl groups, showed a significant negative correlation with adsorption of avemectin (Sun et al., 2012).

In this study, MAC with a Brunauer-Emmett-Teller (BET) surface area greater than 1200 m^2/g was modified with surfactants. The BET surface area of MAC before and after modification was tested using a surface area analyzer. The avermectin loading capacity of modified MAC was compared with other conventional pesticide carriers by analyzing the absorption of avermectin from a methanol solution. Avermectin adsorption data were also modeled using both Langmuir and Freundlich classical adsorption isotherms. Finally, the sustained-release properties and resistance to photodegradation of the delivery system were analyzed and evaluated.

2 Materials and Methods

2.1 Materials

Avermectin was purchased from Qilu Pharmaceutical Co., Ltd. (Inner Mongolia, China). Mesoporous activated carbon and bentonite were obtained from

Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS) and tetrabutylammonium bromide (TBAB) were purchased from J&K Scientific Ltd. (Beijing, China). Kaolin and diatomite were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HPLC grade methanol was purchased from Thermo Fisher Scientific (Beijing, China). Other chemicals were purchased from the Beijing Chemical Factory, China. All chemicals were analytical grade and used as received. The water used in all analytical experiments was Milli-Q water (18.2MΩ.cm, TOC⩽ 4 ppb) prepared using a Milli-Q Advantage A10 system (Millipore, Milford, MA, USA).

2.2 Preparation of the modified MAC with different surfactants

MAC was first purified several times with ultrapure water to remove adsorbed impurities and metal ions. Then it was filtered and oven dried at 100°C for 10 h. Five g of purified MAC was suspended in 500 ml of 10 mmol/L SDS and TBAB solution with stirring at 25°C for 10 h. The mixture was filtered, thoroughly rinsed with ultrapure water to remove excess surfactant, and oven dried at 60°C for 8 h.

2.3 Characterization of MAC

The Brunauer-Emmett-Teller (BET) measurements of MAC and pore structure analysis were conducted at -196.15°C using a surface area analyzer (Tristar Ⅱ 3020, Micromeritics Instrument Co., Norcross, GA, USA). The pore size distribution was calculated with the Barrett-Joyner-Halenda (BJH) method. An elemental analyzer (EA2400, PerkinElmer Inc., Shelton, CT, USA) was used to examine changes in C, H, and N contents using the Pregl-Dumas Method before and after the modification.

2.4 Determination of avermectin content

The avermectin concentration of the suspension was determined by high performance liquid chromatography (HPLC) (Agilent 1260, Agilent Technologies, Ltd., Santa Clara, CA, USA) using a C18 column (5 um, 4.6 mm×150 mm, Agilent Technologies, Ltd., Santa Clara, CA, USA) at room temperature. The mobile phase was composed of methanol and water (90:10). The flow rate was 1.0 mL/min, and a UV detector wavelength of 245 nm was used. The initial concentration of avermectin standard solutions was C_0 (mg/mL). Pesticide-loading capacity (Q_t) was calculated according to the following formula:

$$Q_t = \frac{(C_0 - C_t)V}{m} \qquad (1)$$

where V is the solution volume and m is the mass of adsorbent.

2.5 Modeling of adsorption isotherms

Batch equilibrium studies were carried out by adding 200 mg MAC-SDS into a series of 150 mL Erlenmeyer flasks with 40 mL of an avermectin methanol solution at different concentrations. The flasks were maintained at 25°C for 24 h. After centrifuging at 10,000 rpm for 10 min, the equilibrium concentration of avermectin in the supernatant was determined by HPLC. The amount of adsorbed Av at equilibrium, Q_e, was calculated by

$$Q_e = \frac{(C_0 - C_e)V}{m} \qquad (2)$$

where C_0 and C_e are the concentrations of avermectin at initial and equilibrium stages respectively; V is the volume of the suspension; and m is the mass of MAC-SDS.

2.6 Investigation of sustained release behaviors of Av-MAC-SDS

The release profiles of Avermectin from Av-MAC-SDS samples were investigated as follows: 100 mg Av-MAC-SDS samples were suspended in 20 mL methanol. The suspension was transferred to a dialysis bag. After tightly sealing the dialysis bag it was put into 100 mL methanol as the release medium. The release rate of avermectin from the Av-MAC-SDS sample was calculated by measuring the concentrations of avermectin dissolved in the release medium at different times to evaluate the sustained release property. The concentrations of avermectin were measured using HPLC by collecting 1.0 mL of the release media outside of the dialysis bag at different intervals of 24, 48, 72, 100, 150, 210, and 260 h. Free avermectin was used as a control.

2.7 Photolysis experiments of avermectin in Av-MAC-SDS

The photolytic behavior of avermectin in Av-MAC-SDS was evaluated by the thin-film method described in reference (Crouch et al., 1991), with free avermectin as a control. Ten mL of the methanol suspension of Av-MAC-SDS was placed in several uncovered glass Petri dishes and dried in air at room temperature to form thin films. The glass Petri dishes with thin films were then placed in a Xenon-arc photostability test chamber (XT5409-XPC80, Xutemp Temptech Co. Ltd., Hangzhou, China), at a constant temperature of 25°C. The Petri dishes were removed from the chamber after 24, 48, and 72h. Av-MAC-SDS was then recovered by rinsing the thin films with 5 mL methanol, followed by ultrasonic treatment for 10 min. The suspension was centrifuged and the supernatant was collected and analyzed by HPLC to determine the remaining concentrations of avermectin. The deradation of free avermectin was performed under the same conditions.

3 Results and Discussion

3.1 Characterization of modified MAC

Table 3 shows the BET surface area, total pore volume, and pore size of MAC before and after modification with the cationic surfactant TBAB and the anionic surfactant SDS. The modification did not cause significant changes in average pore size. According to the classification of the International Union of Pure and Applied Chemistry (IUPAC), the pores of adsorbents are grouped into micropore ($d < 2$ nm), mesopore ($d = 2$-50 nm), and macropore ($d > 50$ nm) (Foo et al., 2012). The average pore sizes of MAC before and after the modification were within the mesopore range of the IUPAC classification. The mesoporous structure of MAC-SDS can also be observed from the nitrogen adsorption and desorption isotherms (Marrakchi et al., 2017) in Figure 12. Table 4 shows the C, H, and N contents of MAC before and after modification with surfactants. Compared with non-modified MAC, the C content of modified MAC with surfactants dramatically increased, which indicated that the surfactants had been grafted onto the MAC.

Table 3. BET surface area, total pore volume, and pore size of MAC and surfactant-modified MAC

Carrier	**BET surface area** m^2/g	**Total pore volume** m^3/g	**Pore size** nm
MAC	1232.89	1.08	6.22
MAC-SDS	707.39	0.63	6.19
MAC-TBAB	831.40	0.65	6.10

Table 4. Element contents of MAC and surfactant-modified MAC

Carrier	**C contents** %	**H contents** %	**N contents** %
MAC	69.76	3.02	0.96
MAC-SDS	76.49	3.24	0.91
MAC-TBAB	78.07	3.33	1.14

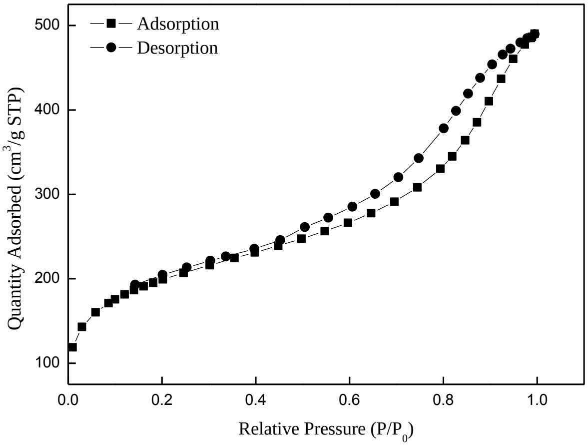

Figure 12. The nitrogen adsorption-desorption curves of MAC-SDS

3.2 Adsorption capacity

The adsorption of MAC for avermectin in solution before and after modification was compared with other commonly used pesticide carriers (talc, bentonite, kaolin, and diatomite). Batch studies were carried out by adding 200 mg absorbents into a series of 150 mL Erlenmeyer flasks with 20 mL of 8 mg/mL avermectin methanol solution. As shown in Table 5 and Figure 13, because of the large special surface area and well-developed pore structure of MAC, the adsorption performance of MAC for avermectin was significantly better than talc, bentonite, kaolin, and diatomite. The adsorption capacity of MAC is mainly related to pore structures and surface chemical properties (Yue et al., 2005), and was improved after modification with surfactants. The non-polar alkyl aliphatic chains of surfactants may enhance the adsorption of MAC for avermectin.

Table 5. The adsorption capacity for Avermectin with different carriers

Carriers	**Amount of adsorbed Avermectin mg/g**
MAC-SDS	275.4
MAC-TBAB	204.4
MAC	156.7
Talc	36.6
Bentonite	35.4
Kaolin	30.7
Diatomite	4.7

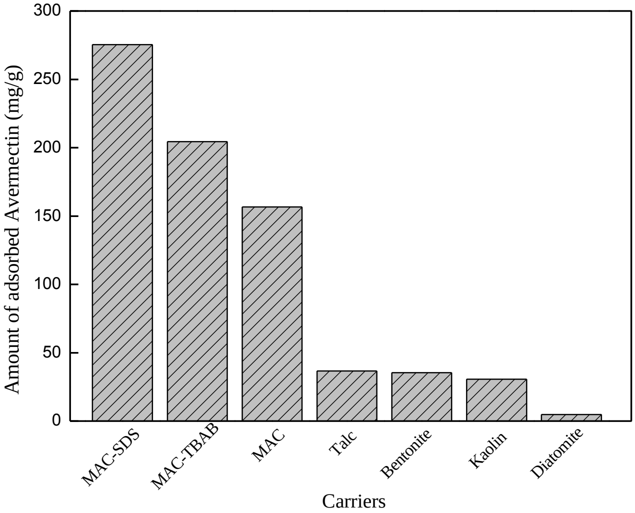

Figure 13. The adsorption capacity for avermectin with different carriers

3.3 Adsorption isotherms

The results obtained for the adsorption of avermectin were examined using Langmuir and Freundlich isotherm models (Langmuir, 1916; Langmuir, 1917; Freundlich, 1906). The correlation coefficient (R^2) was used to evaluate the adequateness of the different models to fit the adsorption process.

The Langmuir isotherm model, which is based on the assumption that the maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, with no lateral interaction between adsorbed molecules, is given by the following equation:

$$\frac{C_e}{Q_e} = \frac{1}{Q_0 b} + \frac{1}{Q_0} C_e \qquad (3)$$

where Q_e (mg/g) and C_e (mg/mL) represent the amount of adsorbed avermectin per unit mass of MAC-SDS and avermectin concentration at equilibrium, respectively; and Q_0 and b refer to the Langmuir constants for MAC-SDS, which are related to the maximum avermectin adsorption capacity to form a complete monolayer on the surface of MAC-SDS and an affinity parameter, respectively.

The Freundlich model is an empirical equation based on adsorption on a heterogeneous surface. It is assumed that the most active sites are bound first and then the binding strength decreases with an increase in the number of sites bound. The Freundlich isotherm is depicted in the following equation:

$$\ln Q_e = \ln K_F + \frac{1}{n} \ln C_e \qquad (4)$$

where K_F and n are the characteristic Freundlich constants that are related to adsorption capacity and intensity, respectively. These parameters can be obtained from the linear plot of $\ln Q_e$ versus $\ln C_e$, which has a slope of 1/n, and an intercept of $\ln K_F$.

The correlation coefficient obtained from the Langmuir model was found to be $R^2=0.9657$ for the adsorption of avermectin on MAC-SDS (Figure 14). For the Freundlich model, the R^2 was 0.5042. These results indicate that the adsorption of avermectin on MAC-SDS can be represented by the Langmuir model.

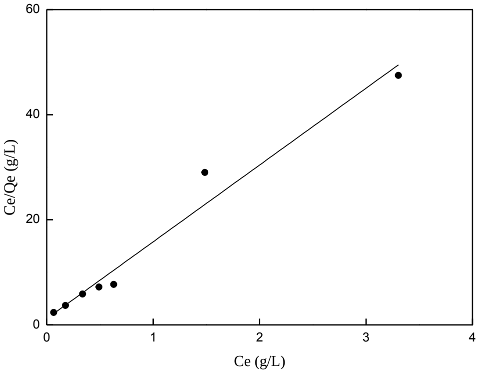

Figure 14. The Langmuir isotherm model of avermectin adsorbed by MAC-SDS

3.4 Sustained release behaviors of Av-MAC-SDS

Figure 15 shows the release behaviors of free avermectin and avermectin from Av-MAC-SDS. Almost the entire amount of free avermectin had been released after 72 h. Compared with free avermectin, the initial burst release of Av-MAC-SDS was not obvious. As expected, Av-MAC-SDS exhibited slower release rates due to the rich pore structure, which slowed the release of avermectin. The release rate of Av-MAC-SDS was relatively fast at the initial stage and then gradually slowed down with increased time, as the avermectin adsorbed on the surface of Av-MAC-SDS was easier to release than the avermectin loaded within the carriers.

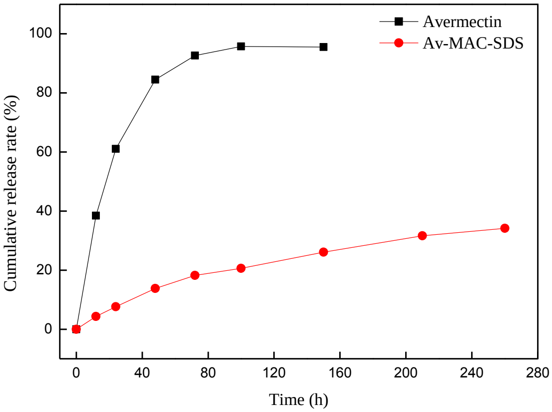

Figure 15. Release profile of avermectin loaded by Av-MAC-SDS

3.5 Effects of Av-MAC-SDS on photodegradation of avermectin

Figure 16 shows the changes of normalized concentrations of avermectin, which are the ratio of remaining concentrations to the initial concentrations of avermectin, under UV irradiation for 0, 24, 48, and 72 h for Av-MAC-SDS and free avermectin, respectively. The photolytic rates of avermectin were 16.9% and 61.4%, respectively, for Av-MAC-SDS and free avermectin after 24 h, indicating that Av-MAC-SDS could protect avermectin from photodegradation. The photolytic rates reached 51.4% and 85.3%, for Av-MAC-SDS and free avermectin, respectively, after 72 h of UV irradiation. The results further confirmed the capability of Av-MAC-SDS for protecting avermectin from photodegradation.

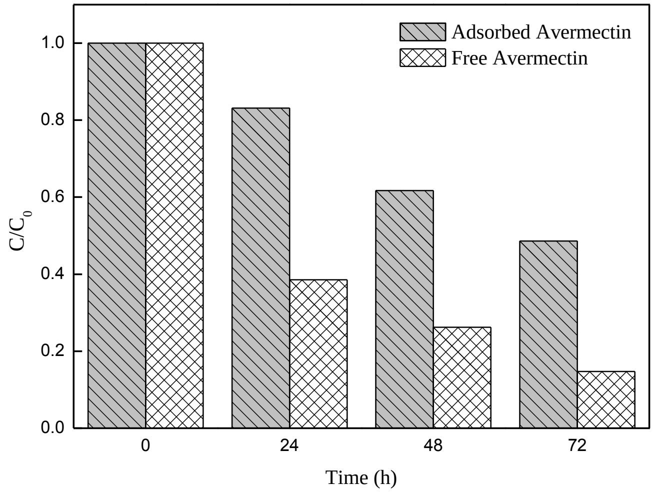

Figure 16. Change in normalized concentration of free and adsorbed avermectin by Av-MAC-SDS to UV irradiation time

4 Conclusion

In summary, surfactant-modified MAC was employed as the carriers for avermectin. The average pore sizes of modified MAC were still within the mesopore range. The surfactant-modified MAC, especially the SDS-modified MAC showed an excellent adsorption for avermectin. The adsorption equilibrium of avermectins by SDS-modified MAC could be fitted by the Langmuir isotherm model. In addition, the MAC-SDS delivery system could significantly improve sustained release of avermectin and also effectively inhibits the photodegradation of avermectin, which is favorable to overcome the environmental sensitivity of biopesticides and improve efficacy in crops protection.

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In last chapter, due to the strong adsorption of mesoporous activated carbon, the loaded avermectin can’t be released totally, which may cause residual risk. Therefore, it’s more favorable to use biodegradable materials as the carriers, and the pesticide can be release completely with the decomposing of the material. Poly lactic acid is biocompatible, biodegradable, and nontoxic materials for drug delivery system approved by the Food and Drug Administration. It has been demonstrated that pesticide delivery system loaded by poly lactic acid could effectively prevent the degradation of sensitive ingredients and prolong the duration of pesticide. It was selected as the nanocarrier of pesticide in this chapter. Abamectin, a mixture of avermectin B1a and avermectin B1b, was used in this work. Tannic acid, a natural plant polyphenol that exhibits adhesive property, was adopted to modified the surface of nanopesticide to increase the affinity to the leaves, which could further improve the efficacy of the nanoformulation. The properties of the novel adhesive nanopesticide were characterized and the efficacy was evaluated.

3.2 Development of Tannin-PEG modified abamectin nano-delivery systems

Abstract: Poor utilization efficiency of conventional pesticide formulation has resulted in overuse, which could increase costs, toxicity to other non-target organisms, concerns about human health and safety, groundwater contamination, causing ecosystem destruction and food pollution. The folia-adhesive formulation is supposed to enhance foliar retention time and utilization efficiency. According to the microstructure of the foliage, the nanopesticides surfaces were modified by affinity groups to improve folia adhesion and decrease the loss from crop foliage. In this study, tannic acid, a bioadhesive natural molecule, has been applied to develop abamectin nanopesticide (Abam-PLA-Tannin-NS) with strong adhesion to foliage by chemical modification. Abam-PLA-Tannin-NS presented better photostability and continuous release behavior. The retention rates of Abam-PLA-Tannin-NS on the foliage was remarkably enhanced by more than 50%, compared with unmodified nanopesticides. Resultantly, the indoor toxicity of Abam-PLA-Tannin-NS was enhanced. The interaction force between tannic acid coating nanoparticles and foliage was mainly from hydrogen bonding. Our findings could be beneficial to develop novel leaf-adhesive nanopesticides with high retention time and bioavailability

Keywords: Nanopesticides; abamectin; adhesive; tannic acid

1 Introduction

Pesticides play an important role in modern agriculture, providing agronomic foundation and economic benefits. However, the improper use of pesticides, such as applying more frequently at higher dosage rates, makes the resistance problems more serious (Huseth et al., 2017; Brown et al., 2017). The redundant usage of pesticides also brings other adverse effects, including increased costs, toxicity to non-target organisms, environmental contamination and risk to humans (Dawkar et al., 2013; Kohler et al., 2013; Talebi et al., 2011). Reducing the use of pesticide and changing among classes of pesticides with different modes of action could help decrease the possibility of resistance. Managing pest resistance is crucial in prolonging the effective period of pesticides.

In the spray application process of pesticides, more than 90% of the pesticides in traditional pesticide formulations fail to target the plant foliage due to droplet drifting, jumping, rolling down, rain washing and decomposition (Nuruzzaman et al., 2016), and the effective durable period on the crop foliage does not provide adequate pest control. Therefore, it is urgent to develop new approaches to improve the pesticide utilization efficiency and control losses. New pesticide formulations are required with advantages of high adhesion capacity on target plants, long duration of efficacy, and low dosage and loss to the environment, so as to decrease the risk of environmental pollution, save labor cost by reducing the application frequency, increasing the safety of the pesticide user, and decreasing the non-target effects when compared with traditional formulations (Zhao et al., 2018).

In the recent decade, the development of nanopesticide formulations has the great potential to improve the performance of pesticides by constructing nano-particle-based delivery systems (Kah et al., 2018a; Kah et al., 2018b; Kah et al., 2019). Due to the small size and large surface area, nano-delivery systems allow regular, precise, long and targeted delivery (Khandelwal et al., 2016), and reduce environmental contamination and exposure to human and other non-target organisms (Pascoli et al., 2018). Moreover, in terms of the crop foliage microstructure, the surface of nanoparticles can be modified easily by affinity groups to improve adhesion and decrease the loss from crop foliage (Zhao et al., 2018). Since chemical modification is expensive, the low-cost natural adhesive products are more practical and favorable for nanopesticides.

Natural adhesive behaviors exist in many living creatures (Lee et al., 2007; Forooshani et al., 2017). These bioadhesive materials are promising for developing site-specific drug delivery systems. A natural adhesive polydopamine-containing material inspired by mussels has been applied in many fields, where catechol groups play a major role in adhesion to various surfaces (Lee et al., 2009; Postma et al., 2009; Cheng et al., 2012; Xiong et al., 2018). Nanopesticides modified with polydopamine exhibited excellent adhesive properties on crop foliage, and enhanced pesticide retention time (Jia et al., 2014; Liang et al., 2018). Due to the extraction process dopamine is complicated and costly, it is not practical for application as a pesticide carrier. As an alternative, a natural polyphenol, tannic acid (TA) that can be extracted from various plants with low cost, has a strong adhesion to various surfaces. TA also exhibits antioxidant, antibacterial and biodegradability properties (Rahim et al. 2014, Shutava et al., 2009). The unique structural properties of TA facilitate the interaction with other materials via electrostatic, hydrogen bonding, and hydrophobic interactions (Rahim et al. 2014). Due to the adhesive properties, TA-modified compounds were employed as coating materials and used for control of bacterial and mammalian cell adhesion, radical scavenging and marine fouling (Ejima et al. 2013; Kim et al. 2015; Sileika et al. 2013).

In this study, polylactic acid (PLA), which is a FDA-approved material widely used as drug/cell carriers in the field of medicine and agrochemicals (Lee et al., 2016; Liu et al., 2016; Yu et al., 2017), was employed as the carrier materials. The final metabolized products of PLA in vivo are carbon dioxide and water, which have no harm to human and the environment. Abamectin nanoformulations that is prepared via emulsion/solvent evaporation methods, and modified with TA were developed and characterized. The mechanism of the improvement on foliage adhesion and enhancement of foliar retention time were investigated in detail.

2 Materials and Methods

2.1 Materials

Abamectin (95.6%) was purchased from Qilu Pharmaceutical Company, Ltd. (Shandong, China). Poly(lactic acid) (PLA; MW approximately 100000) was purchased from Daigang Biomaterial Company (Shandong, China). Poly(vinyl alcohol) (PVA) was purchased from Sigma Aldrich. Dichloromethane (CH_2Cl_2; 99.8%), TA (95%) and poly(ethylene glycol) (PEG; MW approximately 10000) were purchased from J&K chemical company (Beijing, China). The water-dispersible granules (WDG) were purchased from Noposion Agrochemicals Co. Ltd. (Shenzhen, China). All chemicals were directly used as received. The water used in all experiments was Milli-Q water (15 MU cm, TOC # 4 ppb).

2.2 Preparation of nanopesticides

Emulsion solvent evaporation method (O/W) was used for preparing nanopesticides: PLA (40mg/mL) and abmectin (40 mg/mL) were dissolved in CH_2Cl_2 by magnetic stirring to form an organic phase. Polyvinyl alcohol (PVA) (10 mg/mL) was added into ultrapure water to form a water phase. The organic phase was added dropwise to the water phase over 10 min by a shearing machine (C25, ATS Engineering Ltd., Vancouver, Canada) to emulsify, while being cooled in an ice-water bath to prevent the evaporation of CH_2Cl_2. Next, the mixed system was stirred vigorously (1000 rpm) overnight to eliminate all the organic solvent by evaporation at room temperature. The abamectin PLA nanoparticles (Abam-PLA-NS) were prepared.

PEG (12 mg/mL) was added to the Abam-PLA-NS solution and followed by dropping TA (12 mg/mL). Finally, the nanosuspension was centrifuged at 15000 rpm for 10 min at 4°C and the collected pellets were redispersed in deionized water; this process was repeated at least three times to remove as much surfactant as possible. The nanosuspension was lyophilized by a freeze drier (FD-81, EYELA, Tokyo, Japan) to complete the preparation of Abam-PLATannin-NS (Figure 17).

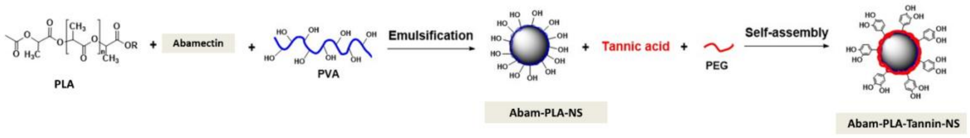

Figure17. Schematic illustration of the preparation for Abam-PLA-NS and Abam-PLA-Tannin-NS

2.3Determination of abamectin loading content

The abamectin loading content (ALC) of nanoparticles was investigated by high performance liquid chromatography (HPLC; 1260 Infinity, Agilent Company, California, USA) using a C18 column (5mm, 4.6mm×150mm, Agilent Technologies; Santa Clara, CA, USA) at room temperature. An appropriate aliquot of nanoparticles was dispersed in CH_2Cl_2 (5mL) and sonicated for 5min, followed by evaporation of the organic solvent at room temperature. Then abamectin was diluted to an appropriate volume with methanol. The mobile phase was composed of methanol and water (90:10). The flow rate was 0.5 mL/min, and the wavelength of UV detector was 245 nm.

2.4 Characterization of the nanoparticles

The hydrodynamic particle size and polydispersity index (PDI) of nanoparticles were investigated by dynamic light scattering (DLS; Zetasizer Nano-ZS90, Malvern, Worcestershire, UK). The average value of three measurements was adopted. A scanning electron microscope (SEM; JSM-7401 F, JEOL Ltd., Akishimashi, Japan) was used to visualize the morphological characterizations of nanoparticles. An aliquot (10 mL) of the re-dispersed nanoparticles was dropped on the surface of a cleaned silicon slice and coated with gold (thickness≤2 nm) after drying at room temperature. The SEM images were recorded at 5 kV and the work distance was 8.5 mm. For TEM, 6 mL of the dispersed nanoparticles was dropped on the surface of a cleaned copper grid. The TEM images were performed at 80 kV and 10 mA after the nanoparticles were completely dried.

2.5 Determination of sustained release behavior of nanoparticles

The sustained release behavior of abamectin nanoparticles was investigated by HPLC. Five mg active abamectin and 10mg nanoparticles were suspended in 5 mL buffer solution (60% methanol solution), respectively, and then the suspension was transferred to different dialysis bags (2000 MWCO), which was sealed into in a brown flask with 95mL 60% methanol solution as release medium. The released abamectin was measured by collecting 5 mL release media outside the dialysis bags at different intervals. The concentration of abamectin dissolved in the release medium was measured with HPLC method described above.

2.6 Evaluation of the photodegradation of nanoparticles

Free abamectin and nanoparticles (100mg each) were irradiated by UV light in a light incubator (XT5409-XPC80, 400W, Xutemp Technic Apparatus Co., Ltd., China). All of the samples rotated around the light at a 10 cm distance and 25°C. Samples (5mg) were collected after 12, 24, 48, 72 and 96 h. The photodegradation behavior of abamectin at specific time intervals was analyzed by HPLC as described above.

2.7 Wettability of nanoparticles on live cucumber foliage

The wettability test was determined based on contact angle. Fresh cucumber foliage obtained from indoor cultivation was carefully selected 21 days after seeding and gently washed using deionized water several times to completely remove dusts on their surface. This foliage cleaning process was carried out very carefully, ensuring that cucumber and foliage surfaces were not damaged. After naturally dried in air, the foliage parts were cut into small pieces and adhered smoothly on glass slides. The aqueous solutions (3μL) containing nanoparticles were slowly added onto the foliage. The measurements were performed with a contact angle (CA) instrument (JC2000D2M, Zhongchen Digital Technic Apparatus Company, Ltd., China). The images of each droplet were taken, and the average value of five measurements was calculated.

2.8 Retention of nanopaticles on live cucumber foliages

The retention test was roughly determined based on SEM images. Fresh cucumber foliage obtained from indoor cultivation was carefully selected 21 days after seeding and gently washed using deionized water several times to completely remove dusts on the surface. Nanoparticles samples (500μL, 3.0 mg/mL) were sprayed on the surfaces of cleaned cucumber foliage at a distance of 15 cm. The treated cucumber foliage was dried for 4 h under vacuum after drying at ambient temperature, and then further SEM measurements were carried out at 3 kV. At the same time, control tests were conducted in which foliage samples after spraying and drying at room temperature and under vacuum were continually washed with deionized water (100 mL).

The adhesive properties of the nanoparticles were evaluated by testing the retention rate after washing. The same amount (C_0) of abamectin in Abam-PLA-NS, Abam-PLA-Tannin-NS, WDG-A, and WDG-B were sprayed onto the clean surfaces of live cucumber leaves without any dust. Each leaf was divided into two equal portions. After natural drying in air, the leaf halves were washed with 100 mL deionized water. The leaves were cut into small thin pieces, and each piece was extracted using a Soxhlet apparatus with CH_2Cl_2 as the solvent for 24 h. The organic phase was collected, and the insoluble solid was filtered off. The obtained filtrate was evaporated slowly under low vacuum at RT to acquire a solid color. To this solid a mixture of CH_3CN, CH_3OH, and H_2O (5 mL, 80:15:5, v/v/v) was added. The mixture was stirred for 1 h and then ultrasonicated for 10 min at RT. After filtering off the insoluble residue, the filtrate was subjected to analysis by HPLC, and the retention amount of abamectin was determined (C_t). The retention rate was obtained by C_t/ C_0. This process was repeated three times, and the average value of retention rate was taken.

2.9 Evaluation of the bioactivity of nanoparticles

Leaf dipping method was used to evaluate the indoor toxicity of abamectin towards peach-potato aphid (*Myzus persicae* L). Abamectin test samples included Abam-PLA-NS, Abam-PLA-Tannin-NS, and two commercially available WDG. Fresh cabbage (*Brassica oleracea* L.) leaves with a diameter of 6 cm were fully immersed in aqueous solutions of 4 kinds of abamectin test samples for 10 s. For each formulation, six concentrations: 0.78125, 1.625, 3.125, 6.25, 12.5, 25 and 50 mg/L, containing 0.1% Triton X-100 were tested. Each treated leaf was air-dried at room temperature and placed in a culture dish; 20 apterous adult aphids were introduced into each dish with a fine brush. The dishes were sealed with microporous plastic wrap and incubated at 75% humidity, 25 °C and 16h:8h (light: dark) cycle. Mortality of aphids was counted after 48h. The regression equation, median lethal concentration (LC_{50}) and its 95% confidence interval were calculated using DPS v12.01 statistical software. Each experiment was repeated four times and the average value was adopted.

3 Results

3.1 Construction and characterization of nanoparticles

The mean size, polydispersity index (PDI) and abamectin loading content (ALC) of Abam-PLA-NS and Abam-PLA-Tannin-NS were summarized in Table 6. The hydrodynamic sizes of Abam-PLA-NS and Abam-PLA-Tannin-NS measured by DLS were 240.7nm and 243.6nm. The result suggested that the surface of Abam-PLA-NS was covered with TA. The PDI of these nanopesticides were less than 0.1, which implied a narrow size distribution and excellent monodispersion. According to the HPLC analytical results, the drug loading content of Abam-PLA-NS was 46.9% and decreased to 38.9% for the Abam-PLA-Tannin-NS, which also suggested that TA was on the surface of nanoparticles. SEM imaging showed that these nanoparticles presented nearly uniform spheres, and the statistical average sizes of 100 nanoparticles from the SEM images were around 150 nm, in good agreement with the DLS results (Figure 18).

Table 6. Mean size, polydispersity index (PdI) and abamectin loading rate (ALR) of nanoparticles

Samples	mean size (nm)	PdI	ALC
Abam-PLA-NS	240.7±1.9	0.03±0.02	38.9 %
Abam-PLA-Tannin-NS	243.6±1.2	0.02±0.01	46.9 %

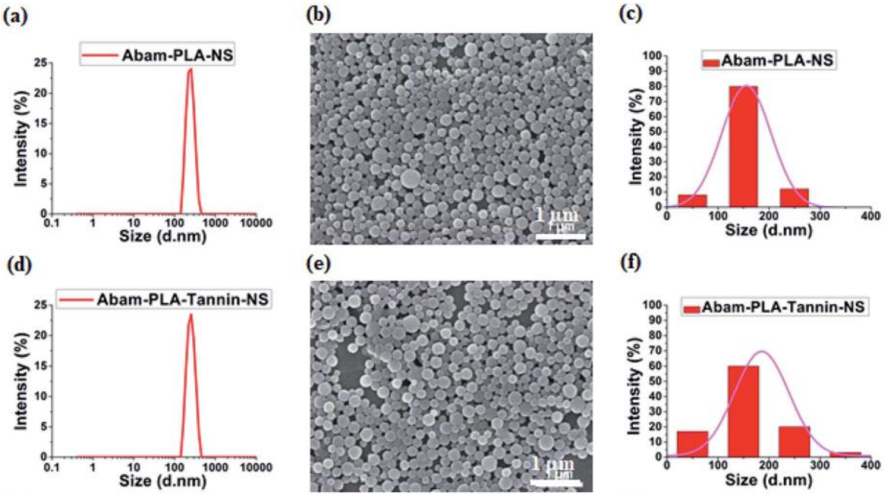

Figure 18. Hydrodynamic size, scanning electron microscopy (SEM) images, and size distributions of Abam-PLA-NS (a–c), Abam-PLA-Tannin-NS (d–f)

3.2 The stability of nanoparticles under different storage conditions

The stability of pesticides is very important, because it affects the shelf life of pesticides. The DLS mean size and PDI were adopted to evaluate the storage stability of these nanopesticides at different temperatures (0 °C, 25°C and 54 °C). The mean size and PDI presented negligible variation at 0 °C and 25°C, indicating that these nanopesticides were very stable at low temperature (Figure 19). However, the mean size and PDI increased at 54 °C, presumably because it is very close to the glass state temperature of PLA (55°C). SEM images showed clearly of these changes (Figure 20). The morphology of these nanopesticides was maintained and the distribution was monodispersed at 0 °C and 25°C. They became broken and aggregated at 54 °C, implying that these nanopesticides were not stable at high temperature.

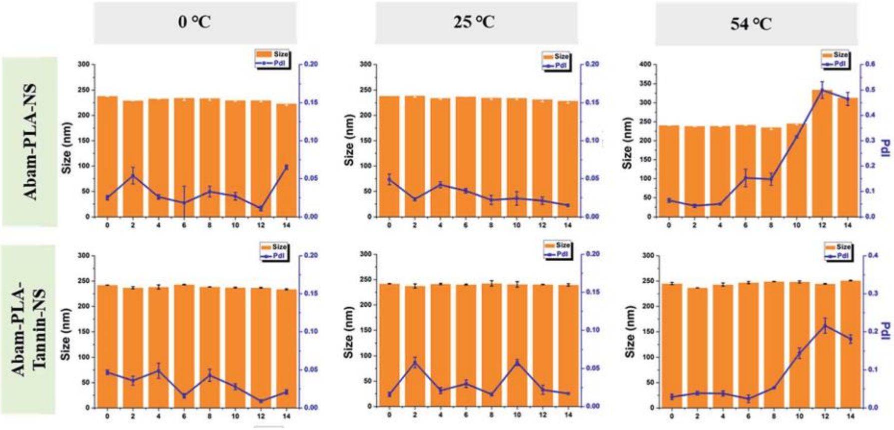

Figure 19. Time dependent variation of DLS mean size and PDI of Abam-PLA-NS and Abam-PLA-Tannin-NS at different temperatures

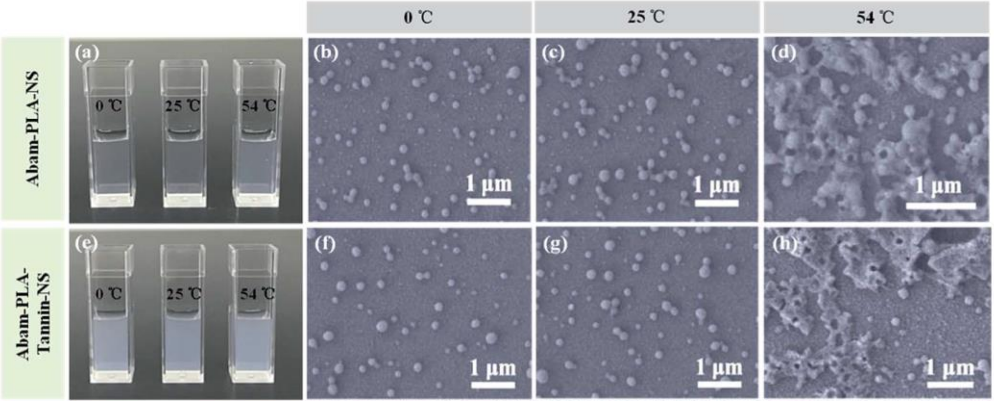

Figure 20. Photographs and SEM images of Abam-PLA-NS and Abam-PLA-Tannin-NS at different temperatures after 14 days storage

3.3 The sustained release properties of nanoparticles

The prepared nanopesticides exhibited sustained release behavior when compared with active abamectin. The sustained release profiles and fitting lines of active abamectin and nanopesticides were presentedin Figure 21. As for active abamectin, the entire amount had been released within 24 h. While the release rates of Abam-PLA-NS and Abam-PLA-Tannin-NS were 43.4% and 41.9% after 21 h of releasing, respectively. The release rates were gradual, with sustained release over > 120 h. These results indicated that PLA-based nanoparticles could prolong the leaching time and increase the utilization efficiency of pesticides, resulting in reduced environmental residues and pollution.

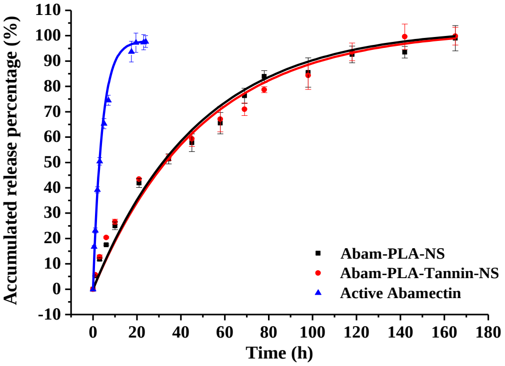

Figure 21. Sustained release profiles of active abamectin and nanoparticles

3.4 Photodegradation properties of nanoparticles

Abamectin is particularl sensitive to ultra violet (UV) light irradiation, and encapsulation was considered as an effective way to improve the photostability. The time-dependent response curves of the photodegradation percentage of active abamectin, Abam-PLA-NS and Abam-PLA-Tannin-NS were illustrated in Figure 22. The photodegradation rate of active abamectin was relatively fast, around 50% decomposing after 48 h of continuous UV irradiation, and more than 80% of active abamectin decomposing after 96 h. In contrast, much lower amounts of the abamectin loaded in the nanoparticles had decomposed within the same time period. These results indicated that the photostability of abamectin loaded in the nanoparticles was significantly improved.

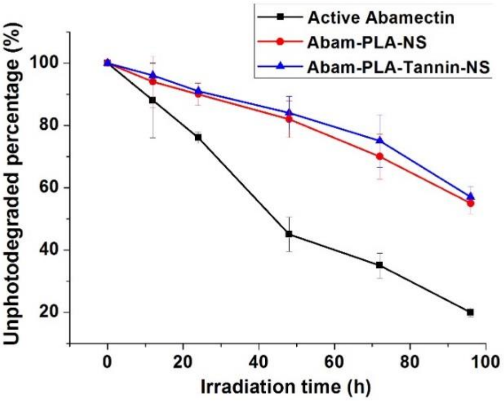

Figure 22. The responsive curves of active Abamectin and Abamectin loaded in nano-delivery system versus irradiated time at 25 °C

3.5 Wettability and retention of nanoparticles on crop foliage

The wettability and retention of pesticides on the surface of crop foliage are important to enhance deposition, adsorption, adhesion and utilization efficiency. It is well known that the rough foliage surfaces have complex microstructure, such as the wax layer, nervure, tomenta and stomata, which influence the wettability and retention properties with external objects. The surface of cucumber foliage has hydrophobic waxy composition that prevents droplets from contacting the surface of foliage. The CA is an essential index to evaluate the wettability of pesticide formulations and the CA optical images on cucumber foliage were shown in Figure 23. The average CA values of Abam-PLA-NS and Abam-PLA-Tannin-NS on the cucumber leaf surface were 93.3° and 91.0°, respectivelyindicating that TA on the surface of nanopesticides could slightly improve wettability on crop foliage.

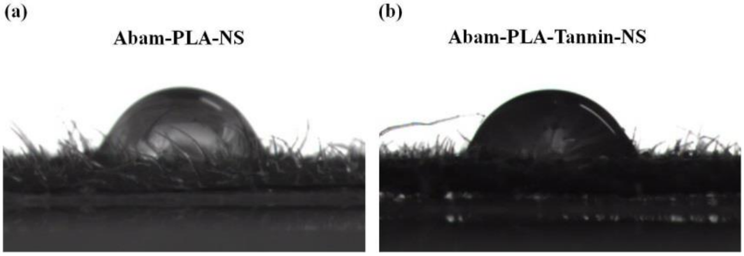

Figure 23. The images of contact angles of abamectin nano-delivery systems on the surface of cucumber leaves

It is very hard to apply pesticides directly on harmful organisms. The crop foliage is the most important medium through which the pesticide activity is available to diseases and pests. The retention time of pesticides on crop foliage is crutial to increase utilization efficiency. The retention time is highly related to the adhesion of pesticides to crop foliage. Here, the retention rate of pesticides by washing was adapted to roughly evaluate the adhesive force and retention time on crop foliage. The calculated retention rates on cucumber foliage were 43%, 67%, 31%, and 27% for Abam-PLA-NS, Abam-PLA-Tannin-NS, WDG-A and WDG-B, respectively (Figure 24). Compared with Abam-PLA-NS, Abam-PLATannin-NS showed much greater affinity to cucumber foliage, with more than 50% enhancement. The TA on the surface of nanopesticides could remarkably increase the adhesion to cucumber foliage, because of the polyphenol groups enhancing the adhesive binding to the crop foliage surfaces. These results are in agreement with previous reports of polyphenol adhesive chemistry (Kim et al., 2015; Du et al., 2016). To better visualize the variation before and after washing in spatial dimensions, SEM was employed to characterize the deposition and retention behavior of nanoparticles on the surface of cucumber foliage. There were many more observed particles of Abam-PLA-Tannin-NS than that of Abam-PLA-NS on the surface of cucumber foliage after washing, confirming that Abam-PLA-Tannin-NS had better adhesion to cucumber foliage than that of Abam-PLA- NS (Figure 25).

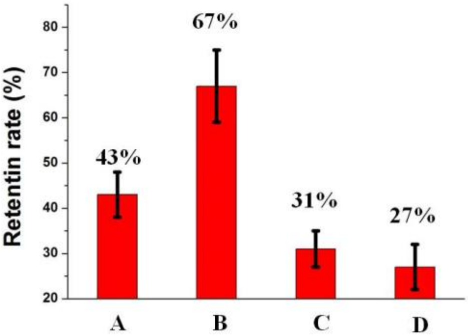

Figure 24. Retention rates of nanoparticles and commercially available formulations determined by HPLC on the surface of cucumber leaves.
A: Abam-PLA-NS B: Abam-PLA-Tannin-NS C: WDG-A D: WDG-B

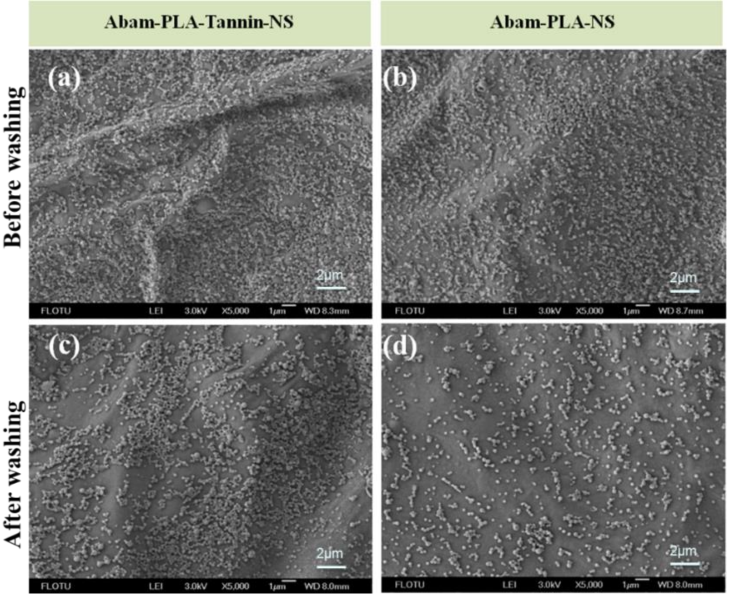

Figure 25. The retention images of nano-delivery system on the surface of cucumber leaves (a) Abam-PLA-Tannin-NS on the surface of cucumber leave, (b) Abam-PLA-NS on the surface of cucumber leave, (c) foliage image with Abam-PLA-Tannin-NS after washing and (d) foliage image with Abam-PLA-NS after washing.

To gain insight into the interaction mechanism between nanopesticides and cucumber foliage, urea (a strong hydrogen bond disrupting agent) was used as washing solvent to evaluate the change in retention rate. The retention rates of

Abam-PLA-NS and Abam-PLA-Tannin-NS decreased in the presence of urea, and they were urea concentration-dependent (Figure 26). The retention rate of Abam-PLA-NS was more sensitive to the urea concentration when compared with that of Abam-PLA-Tannin-NS. The result confirmed that the interaction force between nanoparticles and cucumber foliage was mainly from hydrogen bonding, and Abam-PLA-Tannin-NS showed strong binding to the foliage surface. In addition, there are also possible coordinate bonds between Abam-PLA-Tannin-NS and the crop foliage, because polyphenols can easily coordinate many metal ions. These multimodal bindings between Abam- PLA-Tannin-NS and the foliage surface result in the strong adhesive attraction between them.

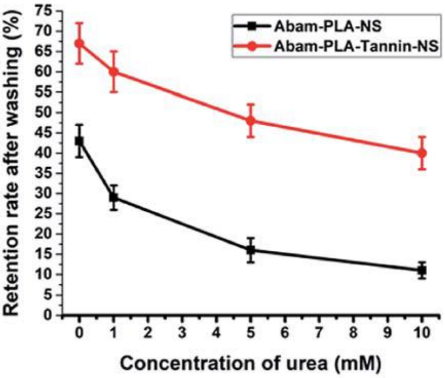

Figure 26. The retention rates effects of different urea concentration on the cucumber foliage surface with Abam-PLA-NS and Abam-PLA-Tannin-NS

3.6 Bioactivity of nanoparticles

The leaf dipping method was used to determine the indoor toxicity of Abam-PLA-NS, Abam-PLA-Tannin-NS and two commercial WDG formulations. The toxicity of pesticides to aphids (*Myzus persicae* L.) was shown in Table 2. The LC_{50} values were 17.38, 10.78, 47.29, and 32.58 mg/mL for Abam-PLA-NS, Abam-PLA-Tannin-NS, WDG-A and WDG-B, respectively. The results showed that two nanopesticides had better insecticidal effects than the other two commercial formulations. In addition, Abam-PLA-Tannin-NS exhibits 1.6-fold higher effect than Abam-PLA-NS, presumably because of higher pesticide retention on the surface of cucumber foliage in the leaf dipping experiment. These results agreed well with the foliar retention results, which indicated that Abam-PLA-Tannin-NS with enhanced adhesion had increased efficacy against their target organisms when compared with the other formulations tested.

Table 7. Indoor toxicity of nano-delivery system and commercial WDGs

Samples	R^2	LC_{50} (mg/mL)
Abam-PLA-NS	0.97	17.38
Abam-PLA-Tannin-NS	0.93	10.68
WDG-A	0.83	47.29
WDG-B	0.92	32.58

4 Conclusion

In this study, Abam-PLA-Tannin-NS with improved adhesion to crop foliage were successfully fabricated by chemical modification on the surface of Abam-PLA-NS using TA. These nanoparticles were spherical with excellent monodispersion. The diameters of the TA-loaded nanoparticles were slightly increased and the drug contents were slightly decreased when compared with their PLA-NS counterparts, implying successful coating with TA. They showed excellent continuous sustained release, and the photostability of abamectin in Abam-PLA-Tannin-NS against UV light irradiation was highly improved. The adhesive force was mainly from hydrogen binding between TA and foliage. The affinitive bindings of Abam-PLA-Tannin-NS to foliage surface resulted in high adhesion and long retention time. Foliar-adhesive nanopesticides could be considered as a resource-saving and environmentally-friendly pesticide formulation, to decrease spraying dosage and pollution in food and the environment.

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Efficacy of the developed nanopesticides

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From Sun, C., Yu, M., Zeng, Z., Francis, F., Cui, H., Verheggen, F., 2019 Laboratory and field evaluation of the biocidal activity of polylactic acid-based nano-formulated abamectin on herbivores and natural enemies. Submitted to PloS ONE

In last chapter, with leaf-dipping method, it has been proved that compared with commercial WDG, nano-formulated abamectin showed higher efficacy on *Myzus persicae*. In order to further evaluate the efficacy of new formulations, a Potter spray tower was used to conduct the bioassay, which was more similar to the field application of pesticide. In this chapter, *Acyrthosiphon pisum* was selected as the target pest and a commercial emulsifiable concentrate, Vertimec, was employed as the positive control. In addition, considering the protection of natural enemies of aphids in agricultural habitats, potential adverse effects on the non-target aphid predators *Adalia bipunctata* were also evaluated. Abamectin has contact poison and stomach poison, so, three different types of exposure were tested. Finally, we performed a field evaluation of the biocidal activity of these formulations on spider mites (*Panonychus citri*).

Laboratory and field evaluation of the biocidal activity of polylactic acid-based nano-formulated abamectin on herbivores and natural enemies

Abstract- Abamectin is a common biocide used for the control of agricultural pests. However, the water insolubility and sensitivity to ultraviolet irradiation of abamectin may result in extra organic solvent introduced in the environment. To solve this issue, it is desirable to develop nanoformulations to encapsulate abamectin with environment-friendly polymers. In this study, the insecticidal activity of polylactic acid-based nano-formulated abamectin was examined on the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae), and the aphid predator *Adalia bipunctata* (Coleoptera: Coccinellidae). The nanoformulation was prepared by emulsion solvent evaporation method and modified by tannic acid. A Potter Precision Laboratory Spray Tower was used to conduct direct spray laboratory bioassays. A comparable insecticidal effect of tannic acid modified nano-formulated abamectin was observed compared to commercial emulsifiable concentrate of abamectin against the aphid. The nano-formulated abamectin was harmless to first-instar larvae of the predator *A. bipunctata*. These results are expected to contribute to the application of nano-formulated pesticides, with further opportunity to develop effective plant protection products which comply with the integrated pest management strategies.

Key words: Nanoformulation, pesticides, abamectin, aphids, lady beetles

1 Introduction

One of the global challenges faced by the agriculture sector is sustainable food production for the rapidly growing human population, reaching 9.7 billion of individuals by 2050 (DESA/UN 2015; Godfray et al. 2014). Therefore, plant protection products and fertilizers are indispensable to maximize the agricultural productivity (De Oliveira et al. 2014). In order to avoid the well-documented deleterious effects of pesticides, the efforts of agrochemical industry are not only focused on looking for new active substances, but also in new pesticide formulations (Villaverde et al. 2017).

During the last two decades, nanotechnology has been considered to have the potential to cause revolution in agricultural practices, especially in agrochemicals (Chen et al. 2011; Kah et al. 2013). The development of nanotechnologies applied to pesticide formulations has facilitated the safe application of conventional pesticides by achieving precise and targeted delivery (Nuruzzaman et al. 2016; Khandelwal et al. 2016). Besides, nanopesticide formulations may decrease the use of organic solvent and improve the biological efficacy of pesticides owing to the increasing dispersity, wettability, and penetration properties (Kah et al. 2013). Among all the nanopesticides, polymer-based nanoformulations are regarded as having the greatest potential for further development and practical application, due to their biocompatibility, biodegradability, modifiability and miscibility (Kah et al. 2014; Campos et al. 2015; Roy et al. 2014).

Abamectin is a mixture of avermectins (around 80% avermectin B1a and 20% avermectin B1b) produced by soil organism *Streptomyces avermitilis*. With a broad spectrum of activity, abamectin is one of most used biocides worldwide to control agricultural pests for its insecticide and acaricide activities (Yu et al., 2017). However, its water insolubility may result in extra organic solvent introduced in the environment (Cui et al., 2018), and it is also susceptible to ultraviolet and strong acidic or alkaline conditions, which may cause premature degradation (He et al., 2013). A great deal of efforts has been made to provide protection and sustainable release of abamectin, among which developing nanoformulations to encapsulate abamectin with environment-friendly polymers is an effective strategy (Li et al., 2016).

Polylactic acid (PLA) is a USFDA-approved polymeric material that is widely employed as drug or cell carrier in the medical area for its biodegradable and mechanical properties that can be adjustable (Lee et al. 2016). Active ingredients (AI) could be protected from photodegradation after encapsulation by PLA nanoparticles (Liu et al. 2016; Yu et al. 2017). Tannic acid (TA) is a plant polyphenol that exhibits antioxidant, antibacterial, antimicrobial, antimutagenic, and anticarcinogenic properties (Rahim et al. 2014). The unique structural properties of TA facilitate the interaction with other materials via electrostatic, hydrogen bonding, and hydrophobic interactions (Rahim et al. 2014). Due to the adhesive properties, TA-modified compounds were employed as coating materials and used for control of bacterial and mammalian cell adhesion, radical scavenging and marine fouling

(Ejima et al. 2013; Kim et al. 2015; Sileika et al. 2013). Therefore, TA modified nanopesticide could improve the adhesion of AI on target crops and pests. Besides, TA could also enhance the dispersibility and biocompatibility of nanomaterials (Zhang et al. 2015), which may result in better efficacy of TA-modified nanopesticides.

In order to evaluate the efficacy and possible side effects of abamectin nanoformulations that is prepared via emulsion/solvent evaporation methods, and modified with TA, laboratory bioassays were conducted to identify the insecticidal effect of nanoformulated abamectin on a major aphid species, *Acyrthosiphon pisum* (Hemiptera: Aphididae). *A. pisum* is a world distributed pest and a vector of more than 30 virus diseases (Hullé et al. 2019). In addition, as the protection of natural enemies of aphids in agricultural habitats remains an imperative issue, potential adverse effects on the non-target aphid predators *Adalia bipunctata* (Coleoptera: Coccinellidae) were also evaluated.

2 Materials & Methods

2.1 Chemicals

Polylactic acid (PLA) was purchased from Daigang Biomaterial Company, China. Abamectin (95.6%) was purchased from Qilu Pharmaceutical Company, Ltd., China. Poly(vinyl alcohol) (PVA) and agar were purchased from Sigma Aldrich. Dichloromethane (CH_2Cl_2, 99.8%), tannic acid (95%), poly(ethylene glycol) (PEG, Mw=10,000) were purchased from Bailingwei Technology Company, Ltd., China. A commercial emulsifiable concentrate (EC) containing 18 g L−1 of abamectin (Vertimec) was obtained from Syngenta, Belgium.

2.2 Nanoformulations

Emulsion solvent evaporation method (O/W) was used for preparing PLA nanoformulation. PLA and abamectin were added to dichloromethane to form an organic phase. PVA was added into ultrapure water to form water phase. The organic phase was added dropwise to the water phase under magnetic stirring and then emulsified. Dichloromethane was eliminated by evaporation at room temperature with magnetic stirring overnight. The abamectin PLA nanospheres (Abam-PLA-NS) were prepared. PEG solution was added into the Abam-PLA-NS, and then 20% tannic acid solution was dropped in the mixture. After stirring for 1h, the mixture was washed with deionized water for 3 times and tannic acid modified abamectin PLA nanospheres (Abam-PLA-Tannin-NS) were prepared.

2.3 Insecticidal effect of nano-formulated abamectin on aphid and lady beetles

To produce aphids, broad beans (*Vicia faba* L.) were used as host plants. The seeds were sown in 30 × 20 cm boxes, which contained a 1:1 mixture of vermiculite and perlite. The plants were infested with aphids at two-leaf stage. Aphids were kept under controlled conditions (22 ± 2 °C, 70 ± 10% relative humidity, with 16 h of light alternating with 8 h of darkness.

A Potter Precision Laboratory Spray Tower (Burkard Scientific Ltd., UK) was used to evaluate the biocidal efficacy of two developed nanoformulations, one abamectin commercial formulation and control solution. For each formulation, six concentrations were tested: 3.125mg/L, 6.25mg/L, 12.5mg/L, 25mg/L, 50mg/L, and 100 mg/L.

An agar solution was prepared to perform the insecticidal assay on Petri dishes (diam. 3.5 cm) containing a plant leave and aphids. Agar powder was mixed with distilled water (1% w/w), heated until boiling and then allowed cooling while constantly mixing. After cooling for approximately 10 minutes, warm agar was poured into each Petri dish to a depth that was at least 3-4mm. A round piece of leave of 33 mm in diameter was cut using a sharpened metal tube, and put on the agar gel with abaxial surface facing skywards. Ten apterous aphid individuals were transferred onto each of the leaf discs using a fine brush.

The bioassay was conducted using Potter Precision Laboratory Spray Tower (Burkard Scientific, Uxbridge, UK) at a spray pressure 0.70 kg/cm2 (69 kPa; 10psi) (Gao et al. 2019). Each aphid-containing Petri dish was sprayed with 1 mL of the tested solution, representing a deposit of 27.9 ± 2.1mg on the leaf-disc. Then, all dishes were sealed with a close-fitting, ventilated lid, and kept at 22 ± 2 °C, 70 ± 10% RH, and a photoperiod of 16:8 (L:D). Two days after the application, the number of alive aphids was counted in each dish. An aphid was considered dead if it failed to react when touched by the brush. There were 3 replicates in each treatment (formulation × concentration).

Lady beetles A. bipunctata were purchased from Biobest Group NV, Belgium. First instars were also tested with both developed nanoformulations and the commercial formulation of abamectin, using the same concentrations. All studies were conducted at 22 ± 1 °C, 30 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

Three groups of insecticidal assays were conducted using lady beetles:

(a) **Direct exposure**: Ten larvae were transferred to a Petri dish and then sprayed with 1 mL of the tested solution. Larvae were then individually transferred to clean plastic Petri dishes and checked for mortality after 5 days (120h). Larvae were provisioned with Ephestia eggs and water until pupation or death.

(b) **Indirect exposure**: Petri dishes were sprayed with 1mL of the tested solution (n=10 for each concentration of a tested solution). A single larva was then individually transferred to a Petri dish. After 24 h of contact, they were transferred to clean plastic Petri dishes and checked for mortality after 5 days (120h). Larvae were provisioned with Ephestia eggs and water until pupation or death.

(c) **Feeding exposure**: 20 aphids (*A. pisum*) were sprayed with 1mL of the tested solution. After air dry, they were transferred to a clean plastic pot where a lady beetle larva was introduced (n=10 for each concentration of a tested solution). Ephestia eggs and water were offered after all the aphids died. Larvae were checked for mortality after 5 days (120h). Larvae were provisioned with Ephestia eggs and water until pupation or death.

2.4 Acaricidal efficacy of nano-formulated abamectin in the field

The acaricidal efficacy of the two nanoformulations were conducted against spider mites (*Panonychus citri*) on a citrus Shiranuhi (*Citrus reticulata* × (C.*reticulata*×C.*sinenesis*)) in Danling County, Meishan, Sichuan Province of China. The trial was carried out according to Chinese national standard "Pesticide – Guidelines for the field efficacy trials (I) – Acaricides against spider mites on citrus" (GB/T 17980.11- 2000). The test trees, with an average age of 20 years, were more than 2m high and the crown diameter was 1.5m. For each tree, 5 different points—east, west, south, north and middle were selected, and 5 leaves with spider mites were labeled at each point. A commercial abamectin water dispersible granule (WDG) was selected as the positive control, and water was used as negative control. The concentrations of the test formulations were 40mg/L, 60mg/L and 80mg/L. For each treatment, one tree was sprayed with 15L. The population of spider mites before spraying, as well as 1 day and 5 days after spraying, were counted.

2.5 Statistical analysis

Insect mortality was corrected by Abbott's formula (1925) taking into account the natural mortality observed on the control:

$$M_c = \frac{M_0 - M_t}{100 - M_t} \times 100$$

Where M_0 is the average mortality and Mt is the natural mortality in the control. Data for LC_{50} values were analyzed by probit analysis (SPSS Statistics). A Chi-square Goodness-of-fit test was used to analyse the data. The dose-mortality relationships were considered valid when the observed data and the expected data did not diverge significantly ($P < 0.05$).

3 Results

3.1 Particle size and morphological characterization of nanoparticles

The mean size (average value of 100 particles) of Abam-PLA-NS by transmission electron microscope (TEM, HT7700, Hitachi Ltd., Tokyo, Japan) was determined to be 150.7±2.2 nm, and it increased to 156.5±2.4 nm for the Abam-PLA-Tannin-NS. These results suggested that the surface of Abam-PLA-NS was capped with TA to form Abam-PLA-Tannin-NS. TEM imaging indicated that these nanoparticles exhibited nearly uniform spheres and excellent monodispersion.

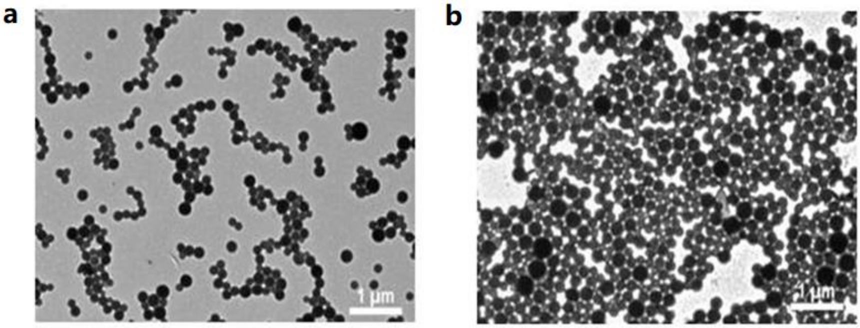

Figure 27. TEM images of Abam-PLA nanoparticles (a) and Abam-PLA-Tannin nanoparticles (b)

3.2 Laboratory insecticidal assay

For all tested formulations, the higher the abamectin concentration, the higher the mortality (Table 8). LC_{50} values were 33.3, 10.1 and 13.1 mg/L for Abam-PLA-NS, Abam-PLA-Tannin-NS, and abamectin EC, respectively. Abam-PLA-Tannin-NS and abamectin EC had similar insecticidal effect on aphids, and both of them had better efficacy than Abam-PLA-NS.

Table 8. Indoor bioassay results of abamectin formulations against aphids after 48h

Formulation	**Toxicity regression equation**	**R^2**	**LC_{50} (mg/L)**	**95% confidence limit (mg/L)**
Abam-PLA-NS	y=2.87+1.40x	0.886	33.3	18.6-59.5
Abam-PLA-Tannin-NS	y=4.11+0.88x	0.812	10.1	4.2-23.9
Abmectin emulsifiable concentrate	y=3.38+1.45x	0.987	13.1	7.50-22.8

The biocidal effects of all tested formulations against lady beetle larvae after 48h and 120h are presented in Table 9, Table 10 and Table 11 for direct exposure, indirect exposure and feeding exposure, respectively. Taking LC_{50} as the evaluation index, two nanoformulations showed higher efficacy than EC in both direct and indirect application 48h and 120h after the spray. While the feeding exposure results showed totally opposite trends.

Table 9. Direct exposure for three abamectin formulations on lady beetle larvae

Formulation	48h				120h			
	Toxicity regression equation	R^2	LC_{50} (mg/L)	95% confidence limit (mg/L)	Toxicity regression equation	R^2	LC_{50} (mg/L)	95% confidence limit (mg/L)
Abam-PLA-NS	y=3.79+1.10x	0.871	12.5	6.2-25.2	y=3.75+1.44x	0.844	7.4	4.2-13.2
Abam-PLA-Tannin-NS	y=3.44+1.26x	0.789	16.6	8.9-31.0	y=3.92+1.37x	0.831	6.0	3.3-11.0
Abmectin emulsifiable concentrate	y=2.72+1.75x	0.935	19.4	12.0-31.3	y=3.36+1.64x	0.877	10.3	6.2-17.1

Table 10. Indirect exposure for three abamectin formulations on lady beetle larvae

Formulation	48h				120h			
	Toxicity regression equation	R^2	LC_{50} (mg/L)	95% confidence limit (mg/L)	Toxicity regression equation	R^2	LC_{50} (mg/L)	95% confidence limit (mg/L)
Abam-PLA-NS	y=2.47+2.26x	0.976	13.2	8.8-19.7	y=3.03+2.01x	0.920	9.7	6.2-15.0
Abam-PLA-Tannin-NS	y=3.52+1.27x	0.983	14.7	7.9-27.3	y=3.40+1.76x	0.960	8.1	5.0-13.6
Abmectin emulsifiable concentrate	y=3.72+0.66x	0.846	83.9	23.5-299.8	y=3.14+1.58x	0.805	15.3	9.1-25.6

Table 11. Feeding exposure for three abamectin formulations on lady beetle larvae

Formulation	**48h**				**120h**			
	Toxicity regression equation	**R^2**	**LC50 (mg/L)**	**95% confidence limit (mg/L)**	**Toxicity regression equation**	**R^2**	**LC50 (mg/L)**	**95% confidence limit (mg/L)**
Abam-PLA-NS	y=2.57+1.16x	0.884	110.2	50.9-238.6	y=2.98+1.09x	0.816	74.0	34.7-157.5
Abam-PLA-Tannin-NS	y=1.63+1.82x	0.998	71.3	42.2-120.4	y=1.13+2.58x	0.984	31.3	21.6-45.6
Abmectin emulsifiable concentrate	y=1.85=1.95x	0.806	41.9	26.4-66.4	y=3.01+1.46x	0.762	22.5	13.0-39.2

3.3 Field acaricidal assay

The reduction rate of spider mites was presented in Table 12. One day after the spray, the commercial formulation showed a slight better reduction rate, but 5 days later, Abam-PLA-Tannin-NS presented a better efficacy than the commercial formulation.

Table 12. Reduction rate (%) of spider mites for the three abamectin formulations

Formulation	**1 day**			**5 days**		
	40mg/L	**60mg/L**	**80mg/L**	**40mg/L**	**60mg/L**	**80mg/L**
Abam-PLA-NS	68.28	75.24	80.13	53.18	65.43	69.49
Abam-PLA-Tannin-NS	72.18	78.28	81.38	59.27	72.78	74.32
Abmectin water dispersible granule	72.12	81.54	85.27	51.18	70.64	70.79

4 Discussion

Nano-formulated pesticides are expected to improve the efficiency of pesticide and reduce environmental pollution. Some studies have confirmed that nanoformulations were harmless to non-target organism, such as different cell lines and soil microorganisms (Pasquoto-Stigliani et al. 2017), but only a few safety studies were carried out on natural predators (Papanikolaou et al. 2018). Nanopesticides may behave differently from conventional pesticide, so it’s necessary to evaluate the efficacy and effect on non-target organisms exactly before field application (Kookana et al. 2014). In this study, the biocidal efficacy of two solvent-free nano-formulated abamectin was tested on aphids and on lady beetles. Abamectin works on glutamate-gated chloride ion channels in arthropods to produce long-term, high-intensity inhibitory effects, causing insects to die. The main action of abamectin is stomach toxicity and contact toxicity. Usually, nano-sized formulations can improve the adhesivity and penetrability of pesticides on surface of organisms (Boehm et al. 2003; Liu et al. 2016). However, Abam-PLA-NS had a higher LC_{50} value and low efficacy than commercial abamectin EC, which could be accounted by the fact that abamectin EC had better dispersion and deposition, because of the existence of organic solvent and other additives. Abam-PLA-Tannin-NS had a similar LC_{50} to the abamectin EC, which attributed to the enhancing dispersibility and contact of abamectin on the epidermis of aphids, due to the properties of TA.

It was reported that abamectin was relatively safe to adult lady beetles (Mani 2018; Ozawa et al. 2016), so larva was selected as the objects. Direct and indirect exposure of nano-formulated abamectin on lady beetles resulted in a similar efficacy as compared to aphids. This suggested that the nano-formulated abamectin had no selectivity for either insect group, but the larvae of lady beetles were more sensitive to abamectin formulations. 120h after spray, both nano-formulated abmectin showed better efficacy than abamectin EC, indicating that PLA as carriers are beneficial to prolong the insecticidal duration and improve the utilization efficiency of abamectin. These results consistent with Kah et al.'s (2013) conclusion that polymer-based pesticide nanoparticles can serve as protective reservoirs and diffusion-controlled release carriers.

For feeding exposure, there was a totally different result. Both nano-formulated abamectin showed lower stomach toxicity than abamectin EC 120 h after feeding. It seems that PLA prevented abamectin from contact with the lady beetles after entering the stomach. The existence of TA also increased the toxicity of abamectin nanospheres due to the improved dispersibility and adhesivity.

In a word, the increasing insecticidal activity of nano-formulated abamectin in combination with the lower stomach toxicity on non-target lady beetles make them suitable as the plant protection products in IPM (Integrated Pest Management) strategies. In addition, the sublethal effects on lady beetles' physiology and behavior should also be considered for a complete analysis of the deleterious effects of nanformulations (Nesneux et al. 2007). Meanwhile, further studies on the field trial would be certainly worthwhile.

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5

General discussion and perspectives

Pesticides are important inputs for preventing major biological disasters and enhancing crop productivity (Wang et al., 2017; Cui et al., 2018). They are conventionally applied to crops by spraying and/or broadcasting (Ahmed et al., 2012). Usually only a very low concentration of AIs, which is much lower than the minimum effective concentration required, has reached the target site of crops and target organisms due to problems such as leaching of AIs, degradation by hydrolysis, photolysis and by microbial degradation. Hence repeated application is indispensable to have an effective control, which might cause some deleterious effects such as soil and water pollution (Nair et al., 2010). Development of environmentally friendly pesticides is a key focus in the agrochemical industry for sustainable agriculture (Yusoff et al., 2016). With the identification of novel active ingredients getting more and more difficult, it is more favorable to develop new pesticide delivery system. The development of nanotechnology could open up novel application in agriculture (Kumar et al., 2019).

The application of nanotechnology is expected to reduce the amount of pesticide needed to assure crop protection, which may be realized by several ways such as by improved apparent solubility, targeted delivery, controlled release, increased leaf adhesion, enhanced bioavailability and improved stability of the pesticide in the environment (Kah et al., 2018). However, nanopesticide cannot be regarded as a single entity (Iavicoli et al., 2017). Different types of nanopesticide have their own intended purposes. Nanoemulsions and nano-dispersion can increase the solubility of poorly water-soluble pesticides, which finally improve the bioavailability of AI while avoiding using a great number of adjuvants (Anjali et al., 2012; Suresh Kumar et al., 2013). Polymer-based and lipid-based nanoformulations aim at slow and controlled release of AI serving as protective carriers and reservoirs (Xiang et al., 2013; Frederiksen et al., 2003). Other nanopesticides that contain inorganic carriers, nano sized metal and metal oxide have also been investigated for slow release and photocatalyzation of the organic ingredients after release to reduce residues in the environment (Wang et al., 2014; Song et al., 2012). So, future research at all levels is necessary to understand their suitable application. Besides, more efforts are needed to overcome shortages of nanopesticides.

Table 13 Advantages and disadvantages of the nanopesticides in this thesis

Advantages	**Disadvantages**
Decreasing use of organic solvent and surfactants;	Hard for up scaling production;
Controlled-release of loaded pesticide;	High-cost of the nanocarrier materials;
Inhibiting the photodegration of pesticide;	Potencial environmental residual risks;
Improving efficiency due to leaf-affinity property.	Potencial exposure risks to human.

1. The function of surfactant in pesticide formulation

There is a thin cuticular membrane, which encloses leaves to protect them from environmental hazards and prevent excessive evaporation of water from the plant surfaces (Guhling et al., 2005; Wang and Liu, 2007; Koch and Ensikat, 2008). Due to the variations of cuticular membrane, waxes, veins, stomata and trichomes, leaves can be classified roughly into two categories: one is easy to wet and the other is difficult to wet (Beattie and Marcell, 2002). Leaves with waxy and hairy surface are difficult to wet, and it is hard to apply pesticide successfully due to the problem of rebounding droplets, which scatter or roll off the leaves after contacting with the plant surface (Massinon and Lebeau, 2013). For effective folia application of pesticide, the wettability of the leaf surface is an important factor in the process of deposition, retention, spread of spray droplets on the leaf surface and the penetration of pesticides into leaves (Zhu et al., 2019). Surfactants are an indispensable part of pesticide formulations, which work on the surface tension of spray droplets at the air–liquid interface and on the contact angle at the liquid–plant interface, and can improve the deposition, retention, spread, penetration and uptake of the spray droplets (Castro et al., 2014; Xu et al., 2011).

In addition to being directly added to pesticide formulations, surfactants are often employed for modification of absorbents, such as mesoporous activated carbon (Malekbala, et al., 2015), aluminum hydroxide (Saitoh et al., 2011), silica (Bryleva et al., 2006), graphene (Liu et al., 2012) and zeolite (Singh et al., 2019). Usually, the surfactant-modified absorbent is used as the remover of contaminants in aqueous solution because of the improved absorption, but some of the absorbents could be developed as the carriers for pesticide (Fernander-Perez et al.,2005; Garrido-Herrera et al., 2006). Previous studies indicate that the absorption of pesticides on surfactant-modified activated carbon provides an effective way to remove the residual of pesticide from the environment (Yin et al., 2007; Bhatnagar et al., 2013). However, absorption characteristics of surfactant-modified activated carbon for pesticide and its effect on protecting pesticide from photodegradation have been rarely studied so far. Our study demonstrated that SDS-modified mesoporous activated carbon could significantly improve sustained release of avermectin and also effectively inhibit the photodegradation of avermectin. But even in the organic solution, the release rate of avermectin was relatively slow, and it is not practical to apply it in the field where the quick action of pesticide against crop diseases needs to be taken into consideration. Therefore, polymer-based nanopesticides that can release AIs with the degradation of polymers is more desirable.

2 The residual concern of adhesive pesticides

Increasing the longevity of spray droplets on leaves means the plants would absorb and uptake more AIs of the pesticide, but once droplets are evaporated completely, leaf absorption of AIs stops, and large crystals come out from the AI residues if the droplets did not spread out on leaves evenly. Crystals may be removed from their impact site by wind and rain, which further reduce the pesticide effectiveness. Therefore, a stable pesticide formulation with a strong binding force to foliage will be efficient for improving residence time on plant surfaces.

There are a lot of living creatures in nature that can create bio-adhesive compounds. These adhesive materials are employed as coating materials and used for control of bacterial and mammalian cell adhesion, radical scavenging and marine fouling. The application of adhesive materials gradually transfers to agriculture in recent years. Plydopamine (PDA), a mussel-inspired polymer has film-forming abilities and adhesive properties. It has been intensively studied as the adhesive coating of pesticide, where catechol groups play a major role in adhesion to various surfaces (Jia et al., 2014; Tong et al., 2017). Tannic acid, a natural polyphenol with the catechol groups, also has a strong adhesion to various surfaces. Besides, TA has a significant effect on the dispersion and mobility of nanomaterials in aqueous environments (Zhang et al., 2015). There are also reports about the preparation of adhesive carrier with synthetic materials (Liang et al., 2018), but research on residual evaluation of these adhesive pesticides is scarce.

Though foliage adhesive pesticide may improve the efficacy, there is also an increasing risk of pesticide residual. Due to the increased surface-to-volume ratio, there is a highly improved reactive surface of nanopesticides. Neglecting the well-documented toxity related to metal or metal oxide nanoparticles, previous studies already showed that the polymer-based nanopesticide with a diameter less than 90 nm could be introduced into the plant from the roots of the rice (Tong et al., 2017). Though some research on residue detection of nanopesticides in crops showed promising results (Zhao et al., 2018; Saini et al., 2015a; Saini et al., 2015b), the process of transfer and dissipation of the adhesive pesticide is still unknown. So, we should focus on the uptake and accumulation of adhesive nanopesticide in crop plants and their translocation to edible plant parts for the next step, and we should be cautious with the application of these nanopesticides before systemic guideline of risk assessment is available.

3 The toxic effect of nanoparticles on human health

Due to their unique size and properties, nanomaterials have numerous applications. With rapid growth of nanoparticles-based products, there is an urgent need to identify their potential toxic effects to the human body. Nanoparticles can get into the human body through various ways, such as skin penetration, inhalation, ingestion, or injection (Li et al., 2015; Sajid et al., 2015). Because of their small size and diffusion abilities, they have the potential to cross the various biological barriers and interact with cells and organs. Particle size, shape, and surface chemistry are key factors that determine performance criteria (Bobo et al., 2016).

It has been proposed that nanoparticles of size less than 10nm act similar to a gas, which can enter human tissues easily and may disrupt the cell normal biochemical environment (Vishwakarma et al., 2010). It is also postulated that particles with size less than 35 nm can penetrate into blood–brain barrier and particles with size smaller than 40 nm can enter into nuclei of cells while those with size less than 100 nm can enter into cells by crossing cell membrane (Oberdörster et al. 2004; Dawson et al. 2009). Particle size also plays an important role in clearance of these materials from the body, with small particles (<10nm) being cleared via the kidneys, and larger particles (>10nm) being cleared through the liver and the mononuclear-phagocyte system (Rolfe et al., 2014; Bobo et al., 2016). Besides, the structure and shape of nanomaterials are two additional vital factors that influence their toxicity. Carbon nanotubes, including single-walled and multi-walled carbon nanotube, showed pulmonary toxicity that included inflammation and rapid onset fibrosis following exposure (Erdely et al., 2016). Surface also plays a role in toxicity, as it influences the adsorption of ions and biomolecules that may change the organism or cellular responses towards particles (Li et al., 2015). Polymeric nanoparticles, such as Poly-(D,L-lactide-co-glycolide), have been reported with least toxicity (Bahadar et al., 2016), however, one report proposed that it was the surface coating induced the toxicity of polymeric nanoparticles towards human-like macrophages (Grabowski et al., 2015).

Research in area of nanoparticles toxicity is very scattered and different toxicity assays have been testd for different kind of nanoparticles. Decisive conclusions cannot be drawn based on available literature. Considering the potential applications of nanoparticles in many fields and to address the knowledge gap, relevant toxic effects of nanoparticles should be assessed by utilizing internationally agreed free of bias *in vivo* toxicological models, targeting the vital systems. Therefore, standard methods must be developed to explore toxicity of all kind of nanoparticles.

4 The effect of nanopesticides on the environment

At present, nanopesticide research mainly focuses on the development of new formulation and evaluation of the efficacy compared with commercial formulations. Studies that investigated the environmental fate of nanopesticide are relatively scarce. The use of nanopesticide formulation may bring benefits, whereas their environmental fate should be well studied in order not to bring further damage to the environment and human health. The possible interactions of nanocarriers in agro ecosystem, their effects on soil microorganisms, pollinators, beneficial insects and other non-targeted organisms should be studied in detail (de Oliveira et al., 2014).

Traditionally, the soluble part of a pesticide has been considered to be important for the transport and bioavailability for degradation. Increasing the solubility of the AI could consequently lead to enhanced mobility and faster degradation by soil microorganisms. Surfactants may also affect the physicochemical properties and fate of pesticide AI in the environment. Pesticides, such as avermectin, that have strong absorption to the soil might become more mobile due to the existence of surfactant modified carriers. The drivers for developing adhesive nanopesticide include reduced application rates as a consequence of reduced pesticide losses from rolling off, leaching and degradation. However, the strong adhesive properties and longer persistence of a pesticide could possibly become a disadvantage. The adhesive formulation may increase contact with operators and residue in the environment. The longer existence of pesticide may arouse resistance of pests and pathogen, negative impact on non-target organisms. Thus, more experiments carried out under realistic conditions are indispensable in order to evaluate whether these effects will have a significant influence on the transport, distribution, and degradation processes of a given pesticide.

5 Perspectives of the research and application of nanopesticides

In recent years, development prospects related to pesticide-controlled release have drawn much attention. Now, it has shown great potential to design and prepare targeted pesticide formulations with environmentally responsive controlled release via compound and chemical modifications (Huang et al., 2018). These environmentally responsive systems are well developed in medicine area and gradually transfer to agricultural sector. Intelligent release in response to environmental agents owns a promising future for revolutionizing agrochemicals. Taking pyraclostrobin as an example, it can exert a positive effect on crops affected by rice blast fungus. However, the highly toxicity to aquatic organisms has limited its application on rice crop. This problem was addressed by BASF in 2016. Seltima, a microcapsule product of pyraclostrobin, permits slow release in a directed manner onto rice leaves based on the humidity sensitivity of the formulation. Therefore, pyraclostrobin can be applied to rice and other crops safely without harming aquatic organisms. The development of nanomaterials and technologies has provided new approaches of creating intelligent nanopesticides. By selecting a suitable and timely administration route, such as environmental light, temperature, humidity, soil pH, and enzyme changes, precise pesticide release can be achieved, which may greatly improve the efficacy of pesticides by reducing waste and pollution (Ye et al., 2015; Xu et al., 2017; Chen et al., 2015; Ding et al., 2014). However, there is still a need for sustained systematic research on environmental responsive pesticide-controlled release formulations, especially in the area of effective response to internal biological stimulation system.

If nanopesticides are regarded as a new kind of “bullet”, we also need a sophisticated “weapon” to launch “bullets” and eliminate the “enemy” in the farm field. Recently, it has been reported that the growing use of unmanned aerial vehicles (UAV) for pesticide application on a wide range of crops gained promising results in East Asia (He et al., 2017). Compared with conventional manual spray applications and classical manned aerial applications, the UAV-based application for agrochemicals is a highly efficient and low-cost alternative. In China, there are several companies offering the services of UAV-based pesticides spray, and low-volume pesticide application at a low flight altitude has been carried out in paddy and corn fields as well as hill lands. However, there are no commercial formulations dedicated to UAV spraying. Only research, development and manufacturing activities are under way.

Traditional solid pesticide formulation easily blocks the centrifugal nozzles of UAV, which would reduce spraying efficiency. While liquid formulation tends to become unstable emulsions after dilution with water, so it must proceed to spray immediately after dilution, which cannot be realized when suffer from bad weather that is not suitable for flight. Thus, new formulations suitable for drone application are necessary. Nanoformulations just meet the need of ultra-low or low volume application through UAVs, with distributing pesticide more evenly for small quantities and controlled releasing. In addition, water-based nanoformulations reducing the drift potentials can also eliminate the environmental impact of harmful solvents and adjuvants.

6 Conclusion

Application of nanotechnology in agriculture is anticipated to enhance the efficacy of pesticides and mitigate adverse impacts of pesticides on the environment and human health. Despite some of the exciting achievement, the current level of knowledge seems to be insufficient for a reliable assessment of the risks associated with the use of nanopesticides. To ensure both the safe use and social acceptance of nanopesticides, comprehensive risk assessment is necessary to provide quantitative predictions of given risks, which enable their evidence-based management. Therefore, more efforts are needed in research and development of nanopesticides for the sustainable agriculture development.

7 References

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Appendix-publications

1. Publications included in the thesis

1) **Sun, C.**, Wang, Y., Zhao, X., Zeng, Z., Cui, B., Shen, Y., Gao, F., Cui, H., 2018. Properties of avermectin delivery system using surfactant-modified mesoporous activated carbon as a carrier. J. Nanomater. 2018, 3038902.

2) **Sun, C.**, Zeng, Z., Cui, H., Verheggen, F. Polymer-based nanoinsecticide: Current developments, environmental risks and future challenges. Submitted to BASE.

3) **Sun, C**., Yu, M., Zeng, Z., Francis, F., Cui, H., Verheggen, F., Laboratory and field evaluation of the biocidal activity of polylactic acid-based nano-formulated abamectin on herbivores and natural enemies. Submitted to PloS ONE.

4) Zhao, X., Cui, H., Wang, Y., **Sun, C.**, Cui, Bo., Zeng, Z., 2018. Development strategies and prospects of nano-based smart pesticide formulation. J. Agric. Food Chem. 66, 6504-6512.

5) Yu, M., **Sun, C.**, Xue, Y., Liu, C., Qiu, D., Cui, B., Zhang, D., Cui, H., Zeng, Z., 2019. Tannic acid-based nanopesticides coating with highly improved foliage adhesion to enhance foliar retention. RSC Adv. 9, 27096.

2. Other publications

1) **Sun, C.,** Wang, Y., Zhao, X., Cui, B., Zeng, Z., Cui, H., 2018. Investigation of poly (lactic acid) nano-microspheres preparation craft. Journal of Agricultural Science and Technology. 20(5), 148-153. (in Chinese).

2) **Sun, C.,** Cui, H., Wang, Y., Zeng, Z., Zhao, X., Cui, B., 2016. Studies on applications of nanomaterial and nanotechnology in agriculture. Journal of Agricultural Science and Technology. 18(1): 18-25. (In Chinese)

3) Wang, A., Wang, Y., **Sun, C**., Wang, C., Cui, B., Zhao, X., Zeng, Z., Yao, J., Yang, D., Liu, G., Cui, H., 2018. Fabrication, characterization, and biological activity of avermectin nano-delivery systems with different particle sizes. Nanoscale Res. Lett. 13, 2.

4) Yu, M., Yao, J., Liang, J., Zeng, Z., Cui, B., Zhao, X., Sun, C., Wang, Y., Liu, G., Cui, H., 2017. Development of functionalized abamectin poly (lactic acid) nanoparticles with regulatableadhesion to enhance foliar retention. RSC Adv. 7, 11271.