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基于纳米技术的防治小菜蛾的农药新剂型研究

Study of New Insecticide Formulations Based on Nanotechnology for Controlling *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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A DISSERTATION FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

Agricultural Entomology and Pest Control

(Nanotechnology & Development of Insecticides)

By

Ali Ahmed Zaky Shoaib

Supervised by

Professor Zuhua Shi

Institute of Insect Sciences, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, P.R. China

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I hereby declare that this dissertation is my original work carried out under the guidance and supervision of Professor Zuhua Shi, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China and has not been submitted for examination to another University or any other institution of higher learning for the award of degree.

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This work is dedicated to

My beloved Mom, and Dad (May Allah bless his soul rest in peace), my sincere brothers, my dear teachers in College of Agriculture and Biotechnology, Zhejiang University, and to my all dear friends who have always remembered me in their prayers, sacrificed, encouraged and supported me.

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Contents

Abstract

The chemical pesticides are useful for protecting the crops from insect damage during the growing season. The use of higher dosage and repeated applications of conventional pesticides have led to the rapid development of insect resistance to pesticide and adverse effects on human health and environment. Accordingly, researchers are prompted to identify an alternative insecticidal agent for crop protection. Nanocides are being considered as alternatives to conventional insecticides because they are expected to lessen the application rate and reduce the chances of resistance development in pests. Colloidal delivery systems have been widely used as carriers for controlled delivery of pesticides to improve the efficacy and photostability of natural and semi-synthetic pesticides. In this thesis, I carried out two experiments, and obtained the results as following.

In the first experiment, I evaluated the insecticidal effects of nanosilica on larvae of *Plutella xylostella*, in a laboratory by using dust spray, larva dipping, leaf dipping, and solution spray methods. Dust treatment showed a more highly significant effect than the other three treatments. The mortality percentage increased up to 58 % and 85 % at 24 and 72 h after treatment, respectively, when nanosilica was applied at a rate of 1 mg cm^{-2}. In all four bioassays, mortality rate increased with both increased time after nanosilica exposure and increased concentration. Light microscopy and scanning electron microscopy images showed that larval death was due to desiccation, body wall abrasion, and spiracle blockage.

In the second experiment, I prepared emamectin benzoate nanoformulations (EB+NFs) depending on colloidal delivery systems such as polymeric nanocapsules (PN) and two types of the nanosilica, mesoporous nanosilica (MCM-48) and silicon

dioxide nanoparticles (SNPs) as carriers for the emamectin benzoate (EB). The nanoformulations thus formed were characterized by using X-ray diffraction (XRD), fourier transform infrared (FTIR) spectoscopy, particle size, zeta potential, morphology, absolute recovery (AR), entrapment efficiency (EE), UV stability and release kinetics. The obtained results showed that the carriers had a remarkable loading ability for emamectin benzoate and improved the emamectin benzoate photostability. The entrapment efficiency of nanoformulations were 92.84 %, 87.45 % and 71.19 % for emamectin benzoate polymeric nanocapsules (EB+PNC), emamectin benzoate SNPs (EB+SNPs) and emamectin benzoate MCM-48 (EB+MCM-48) respectively. The insecticidal activity of (EB+NFs) against larvae of *P. xylostella* in third instar showed that the (EB+SNPs) was the most effective than other two EB+NFs and EB alone. The LC50 values were 0.18, 4.03, 8.49 and 11.06 mg L^{-1} for EB+SNPs, EB+MCM-48, EB+PNC and EB alone, respectively.

The obtained results above can suggest that the colloidal delivery systems that used in this study could improve the efficacy and photostability for emamectin benzoate and they are able to overcome the disadvantage of the natural and semi-synthetic pesticides such as environmental sensitivity and increase the efficacy of insecticides, which eventually leads to reduce the dosage of pesticides needed, reducing the number of applications required in comparison to conventional formulations.

中文摘要

Abstract in Chinese

在农作物种植季节，化学农药在保护作物免受害虫为害上起着重要作用。然而，农药的高剂量使用和反复使用也导致昆虫对农药产生抗药性，对人类健康和环境产生不利影响。因此，这些促使研究人员寻找替代性的杀虫制剂来保护农作物。当前，越来越多的考虑纳米杀虫剂来替代传统的化学杀虫剂。因为纳米杀虫剂有望减少农药的使用率和害虫产生抗药性的机会。胶体输送系统正广泛地用作缓释杀虫剂的载体来改善天然和半合成杀虫剂的效率和光稳定性。本论文开展了二方面的研究，获得了如下结果：

首先，制备并用喷粉法、幼虫浸渍法、叶片浸渍法和喷雾法评价了纳米二氧化硅颗粒对小菜蛾三龄幼虫的杀虫效果，结果表明喷粉法处理比其他三种处理方法具有更高的杀虫效果，当纳米二氧化硅以 1mg·cm-2 的剂量喷粉处理时，处理后 24 的幼虫死亡率可以达到 58%，而处理后 72 小时的幼虫死亡率可达到 85%。四种处理方法，幼虫死亡率均随着处理后时间的延长而升高，随着处理剂量的升高而增加。光学显微观察和扫描电镜观察结果表明，幼虫失水、体壁磨损和气门阻塞是幼虫死亡的原因。

然后，利用乙基纤维素、硅酸盐制备了乙基纤维素纳米胶囊（PNC）、二氧化硅纳米颗粒（SNPs）和介孔二氧化硅纳米颗粒（MCM-48），利用它们作为载体负载甲氨基阿维菌素苯甲酸盐（EB），制备了三种纳米制剂（EB+NFs），通过 X 射线衍射分析、傅里叶变换红外分光光度分析，测定胶囊或颗粒大小、Zeta 电位、形态、绝对回收率（AR）、包封率（EE）、对紫外线的稳定性及释放动力学等评价三种纳米制剂。最后测定了三种制剂和未包

封 EB 对小菜蛾 3 龄幼虫得的杀虫活性。结果表明，三种纳米载体具有显著的 EB 负载力，能改善 EB 的光稳定性，EB + PNC、EB + SNPs 和 EB + MCM-48 的包封率分别为 92.84%，87.45%和 71.19%。对小菜蛾三龄幼虫的杀虫活性以 EB + SNPs 为最好，EB + MCM-48 为次之，EB + SNPs 和 EB + MCM-48 的 LC50 值分别为 0.18 和 4.03，EB + PNC 和未包封 EB 的 LC50 值分别为 8.49 和 11.06mg L-1。

综合以上结果，本研究所用的胶体输送系统可以提高 EB 的效率，改善 EB 的光稳定性，它们能够克服天然和半合成农药对环境敏感性的不足之处，同时增加杀虫剂的效率。这样与传统制剂相比，最终将导致杀虫剂应用次数减少，所需剂量降低。

CHAPTER 1.

Introduction

Insects are the biggest animals in population size that can be found in all kinds of possible environment around the world. They have successful evolutionary history. Their successful existence can be attributed to many important evolutionary aspects like habits diversification, wings, metamorphosis, malleable exoskeleton, desiccation-resistant eggs and high reproductive potential (Perlatti et al. 2013). The insect pests can have adverse and damaging impacts on agricultural production and market access, the natural environment, and our lifestyle. Pest insects may cause problems by damaging crops and food production, parasitising livestock, or being a nuisance and health hazard to humans. More than 500,000 species of insect feed on the plants among the all identified insects. About 75 % of them have a specific diet, eating only a limited range of species (Chapman 2009). About 10,000 insect species are plagues and, attacking the food production, either in the field or after the harvest (Ware and Whitacre 2004). It was estimated that somewhere around 14-25 % of total agriculture production is lost to pests yet (DeVilliers and Hoisington 2011). In order to avoid huge economic losses of important crops that are caused by insect pests, several kinds of chemicals have been used to manage them either by killing or by inhibiting their feeding and reproduction behavior. These chemicals are known as insecticides. The usage of insecticides is founded since ancient times, with literature providing key evidences from the 16th century BC. Several chemicals and organic substances were used for the management of harmful insect pests such as fleas and gnats (Panagiotakopulu et al. 1995).

Nowadays, the pesticides are widely employed around the world in agriculture sector for control of insect pest to produce abundant food supply for a growing population. The estimated production of pesticide chemicals and other biological products is about 2 million metric tons and their worldwide annual budget is US$35 billion dollars (Ghormade et al. 2011). Their indiscriminate use has led to several problems, including environmental pollution, serious health hazards to humans and animals, pest resistance to pesticide, destruction of beneficial insects. Due to these environmental concerns, efforts have been raised to minimize pesticide environmental risks by developing new and modern approaches for management of insect pests. In the recent years, natural and semi-synthetic pesticides have gained interest as a promising alternative for conventional pesticides to insect control (Forim et al. 2013). The disadvantages of these pesticides include activity and persistent under various environmental conditions such as sunlight, humidity and rainfall.

From the past few decades, nanotechnology has been growing rapidly around the world. It showed considerable promise for use in crop and foodstuff protection. Nanomaterials (NMs) are being preferentially harnessed because they offer a greater surface area and circulate more easily. Nanomaterials play a vital role in the production of novel pesticides formulations such as metallic oxide nanoparticles, nanoemulsions, nanocapsule and nanosuspension. They enhanced the efficacy of pesticides and reduced the side effects on the environment compared to traditional pesticide formulations. Lot of studies proved the effectiveness of nanomaterials e.g., Stadler et al. (2010) reported that alumina nanoparticles have toxic effects against two stored grain species, *Sitophilus oryzae* and *Rhyzopertha dominica*. Goswami et al. (2010) used different kinds of nanoparticles, viz. silica nanoparticles (SNPs), zinc oxide (ZNP), aluminium oxide (ANP) and titanium dioxide (TNP) for the

management of *S. oryzae.* They found that SNPs and ANP were highly effective than ZNP and TNP, and that the different insecticidal effect exists among different surface functionalized spherical SNPs. Recently, few other studies also showed the entomotoxic effects of silica nanoparticles against the different insect pests such as it proved effective against *S. oryzae* (Debnath et al. 2011), *Callosobruchus maculates* (Rouhani et al. 2012; Arumugam et al. 2016), *Corcyra cephalonica* (Vani and Brindhaa 2013). Debnath et al. (2012b) reported that the amorphous nanosilica (SNPs) could effectively kill the *Spodoptera lecture* larvae at a dosage of 0.5 mg cm^2. According to these results, Debnath et al. (2012b) suggest that the amorphous nanosilica can be used as a novel insecticide in the agriculture sector (Hill and Foster 2000; Gu et al. 2015).

Several pesticides are sensitive to UV-light and their half-life time is very short, such as avermectin (6 h) and phoxim (40 min) … etc. The emamectin benzoate is a novel semi-synthetic derivative of the natural product abamectin of the avermectin family of 16-membered macrocylic lactones. This epi-methyl amino derivative showed increased effectiveness against a broad spectrum of lepidopterous and coleoptera pests with application rates ranging between 8.4–16.8 g-a.i. ha^{-1} (Jansson et al. 1997; Krämer and Schirmer 2007). In fact, the avermectin compounds are degraded rapidly after application, due to their photolysis and shorter half-life after application in field. In addition to this, these compounds are rapidly degraded by soil microorganisms in the agriculture field (Krämer and Schirmer 2007). Unfortunately, the commercial formulation of emamectin benzoate is sensitivity to the light and temperature. These problems limit the use of emamectin benzoate in agriculture sector because an insecticide should persist in field for longer time to achieve good control of pests.

Therefore, it is necessary to encapsulate the active ingredients into some protective form to avoid from photo-degradation (De et al. 2014).

Colloidal delivery systems such as polymeric (nanocapsules and nanospheres) have been used to overcome these disadvantages and to improve the insecticidal properties to protect the crops from insect damage according to the principle of controlled release formulations (CRFs). Colloidal delivery systems show high efficiency as a means of efficiently delivering one or a mixture of active ingredient to their site of action. Furthermore, Nanoparticles (NPs) can reduce the side effects of the insecticides and improve the stability or photostability of active ingredients (Charcosset et al. 2005). Now a day, CRFs technology have been proved very effective and best approach for solving the problems that are associated with the application of agrochemicals. Some new advances have been achieved in the utilization of polymers for controlled release formulation of agrochemicals, such as fertilizers, pesticides, growth regulators. Control release formulation of pesticides is an important way through which effectiveness of agricultural chemicals can be maintained for the targeted insect species. Thus, the basic purpose of CRF is to provide protection to the active agent during the supplying process, maintain the active agent real concentration within optimum range over a specified time and to allow the release of active agent to the target. Smaller quantities of CRF active agents produced a significant increase in the specificity and persistence of biocides and they reduced the side effects of chemical losses that are caused by leaching, degradation and volatilization. Polymers macromolecular nature is very important to control chemical losses and serves primarily to control mobility, delivery rate and effectiveness of the active components time (Akelah 2013).

A lot of work have been done on colloidal delivery systems in agricultural sector, such as polymeric acephate nanocapsule, which was synthesized by polyethylene glycol (PEG-400), showed increase solubility in the water, great efficiency at a lower dose and also reduces acephate toxicity to non-target organisms in the agricultural fields and increases the stability, moreover, showed lower the economic cost, when compared with the commercial acephate formulation (Choudhury et al. 2012; Pradhan et al. 2013). Also, Yang et al. (2009) found that polyethylene glycol (PEG) coated NPs loaded with garlic essential oil are efficient against adult *Tribolism castaneum*. The control efficacy against adult *T. castaneum* was remained over 80 % after five months with NPs but was only 11 % without NPs. The emamectin benzoate microcapsules based on a copolymer matrix of silica– epichlorohydrin–carboxymethylcellulose could protect emamectin benzoate against photo- and thermal degradation effectively and thereby increase their efficacy against agricultural pests, reducing the risk to the environment and human health (Guo et al. 2015). On the other hand, the preparation of porous hollow silica nanoparticles (PHSNs) with various shell thicknesses in the range of 5–45 nm, and a pore diameter of about 4–5 nm is being been described for the same purpose. PHSNs have been synthesized by a sol-gel route with two different structure directing templates, and their shell thickness has been controlled by adjusting the reactant ratio of sodium silicate/calcium carbonate. PHSNs can protect the model pesticide avermectin against photo-degradation effectively (Sayyed and Wright 2006; Li et al. 2007). The UV-shielding property can be further improved by increasing the shell thickness. The porous hollow silica nanoparticle (PHSN) improved the performance of controllable release, photo-stability and water solubility of abamectin by changing the porous structure of silica nanoparticles, which is favorable to improve the bioavailability and reduce the residues of pesticides (Wang et al. 2014). In another study, it was found

that the controlled release nanoparticulate systems based on mesoporous silica nanoparticles (MSNs) were successfully used to adsorb and release imidacloprid (Popat et al. 2012).

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive pests of cruciferous crops worldwide; particularly cabbage, broccoli and cauliflower especially in Asian countries (Talekar and Shelton 1993; Sarfraz et al. 2006). First instar larvae of DBM enter into leaf parenchyma tissues. It feeds between the upper and lower side of leaves and create mines in the leaves. Second and third instar larvae feed on the leaves and damage the leaf tissues. They make transparent windows in the leaves. Fourth instar is very voracious, and it chews the leaves from the both sides. Its life cycle is relative short as it completes whole life cycle around 18 days and its population may increase up to 60 folds (De Bortoli et al. 2013). Previous studies showed that DBM have strong flying mechanism. It can continuously fly for several days and it can cover distances up to 1000 km per day, but its survival rate under the low temperatures and high altitude is still unknown (Talekar and Shelton 1993). The average management expenses for DBM has been estimated at 4~5 billion US$ (Zalucki et al. 2012). Also, it has short generation time, genetic plasticity, high fecundity and particularly the intensive selection pressure (Jiang et al. 2015), it was the first insect pest reported to develop resistance against dichlorodiphenyltrichloroethane, shortly after 3 years of the chemical use (Angkersmit 1953). This insect also subsequently showed significant resistance to most insecticides, including recently introduced compounds with new modes of action, such as spinosad, avermectins, indoxacarb, the bio-pesticide *Bacillus thuringiensis* cry toxins, and the anthranilic diamide chlorantraniliprole (Li et al. 2006; Sayyed and Wright 2006; Zhao et al. 2006; Pu et al. 2010; Troczka et al.

2012; Wang and Wu 2012; Hu et al. 2012; Sukonthabhirom and Siripontangmun 2013; Wang et al. 2013).

Aim of this study is.

1. Synthesis and evaluation of the insecticidal properties of nanosilica (SNPs) as an alternative to conventional pesticides against diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae), in addition, to find the best application method for nanosilica to control this pest.
2. Preparation and characterization of emamectin benzoate nanocapsules depend on colloidal delivery systems to improve the emamectin benzoate formulation and stability under various environmental conditions such as sunlight, humidity and rainfall.

CHAPTER 2.

Review of Literature

Nanotechnology considered a broad interdisciplinary area of research, development, and industrial activity that has been growing rapidly worldwide for the past decade. In the past decade, nanotechnology provided a wide range of novel pesticide formulations such as nanoemulsion, nanocapsule, nanosuspension, and metallic oxide nanoparticles (NPs), with higher efficacy on pest control and lesser harmful effects on the environment compared with the traditional pesticide. This technology aims to reduce the indiscriminate use of conventional pesticides and ensure their safe application. This review investigates the potential of nanotechnology especially the application of nanotechnology in pesticide formulations.

2.1 Solid nanoparticles as nanopesticides

Previous studies have confirmed that metal NPs can be effective against plant pathogens, insects, and pests. Hence, NPs can be used in the preparation of new formulations such as pesticides, insecticides, and insect repellants (Barik et al. 2008; Owolade and Ogunleti 2008; Gajbhiye et al. 2009; Goswami et al. 2010). Bhattacharyya et al. (2010) described the importance of nanotechnology in the agricultural field including the management of harmful insect pests in the future and can also accelerate the green revolution into next two decades. Lot of studies proved the effectiveness of nanomaterials e.g., Barik et al. (2008) reported that the basic mechanism of nanosilica as nanopesticides for the management of insect pests. He revealed that insect pests protect their body water through cuticular lipids. Nanosilica absorbed water from the cuticular lipid layer and created desiccation that ultimately

led to death of the insect. Surface charged modified nanosilica (~3–5 nm) could be successfully used in the field of agriculture and veterinary for the management of insect pests and ectoparasites (Ulrichs et al. 2005). United States Department of Agriculture (USDA) has declared the commercial use of amorphous silica is safe (Stathers et al. 2004). On the other hand, Stadler et al. (2010) develop nanostructured alumina (NSA) insecticides for the management of insect pests. This NSA was proved to be helpful for the management of grain pests, *Sitophilus oryzae* and *Rhyzopertha dominica*. He also compare effectiveness of NSA formulations with the diatomaceous earth (DE) formulations and proved that NSA is more effective than DE (Stadler et al. 2012). Goswami et al. (2010) evaluated the control effect on *S. oryzae* of the different kinds of NPs, namely, silica nanoparticles (SNPs), aluminum oxide nanoparticles (ANP), zinc oxide nanoparticles (ZNP), and titanium dioxide nanoparticles (TNP). They found that SNPs and ANP were considerably more effective than ZNP and TNP, and that the different insecticidal effects were existed among various surface functionalized spherical SNPs.

The entomotoxicity of SNPs was tested against rice weevil, *S. oryzae*, by Debnath et al. (2011) who found that amorphous SNPs were highly effective against this insect pest, causing more than 90 % mortality, indicating the effectiveness of SNPs to control insect pests. Also Debnath et al. (2012b) indicated that the amorphous SNPs could effectively kill the *S. litura* larvae at a dosage of 0.5 mg cm^{-1}. Based on the results, they suggested that amorphous nanosilica can be used as a novel insecticide by the agricultural sector. Alos, Song et al. (2012) loaded dispersible SNPs with insecticide chlorfenapyr and found it increased biological efficacy when compared to microsized chlorfenapyr. They attributed this to both the nanosize and the intrinsic pesticidal action of the SNPs themselves. On the other hand, Rouhani et al. (2012) used silver nanoparticles (AgNP) and silica nanoparticles (SNPs) against the larvae

and adults of *Callosobruchus maculatus* on cowpea seed. The bioassay results showed that the LC_{50} value for AgNP and SNPs nanoparticles against the adult were 2.06 and 0.68 g kg^{-1} whereas against the larvae were 1.00 and 1.03 g kg^{-1}, respectively. The both nanoparticles AgNP and SNPs have been proved to be highly toxic to the adults and larvae of *C. maculatus* with 100 % and 83 % mortality, respectively. These results suggested that the SNPs and AgNP can be used for the pest management programs of *C. maculatus*. In addition to, Arumugam et al. (2016) used hydrophobic silica nanoparticles (SNPs) damaging pulse seeds including *Vigna unguiculata*, *Macrotyloma uniflorum*, *Cajanus cajan*, *Vigna mungo*, *Cicer arietinum* and *Vigna radiate* to control the stored pulse beetle, *Callosobruchus maculatus*, SNPs severely effected the life parameters of *C. maculatus* by reducing the oviposition period and adult emergence. A complete growth retardation was noticed in *C. maculatus* feeding on the *C. cajan* seeds. SNPs showed positive effects on the treated seeds of these varieties. No harmful effect was noticed on the growth of seeds and growth rate of root and shoot. Similarly, the soil microflora was remained unaffected from silica nanoparticle treatments. These positive aspects of silica nanoparticles provide strong support to the usage of these nanoparticles for the management of *C. maculatus.* Recently new approaches are using in the development of NSA dust with the help of glycine-nitrate combustion process which has been proved to be very effective for the management of S. oryzae than R. dominica (Buteler et al. 2015).

2.2 Nanopesticide Delivery Systems

2.2.1 Polymer-Based Nanopesticide Delivery Systems

Pesticides are very harmful for the human beings and pollinating insects but nanomaterials can decrease the toxicity and increase the efficacy of these pesticides (Mousavi and Rezaei 2011), New formulation of nanopesticides can increase the solubility power of weak soluble active ingredients and help in releasing the active ingredient slowly. Bioavailability capacity of poorly water-soluble agrochemicals can be enhanced through the usage of different additives or by nanparticulate formation (Kah et al. 2013).

Polymeric substances are rapidly used in agro-based industry in controlled-release formulations of agrochemicals. Controlled release was defined as use of polymer containing substances of agricultural properties which are released into the field of interest at constant rates over prolonged periods of time to prevent reagents from the danger of being washed away by irrigation and rain. Polymers are used either as encapsulation membranes for reagents or as supports to chemically attached agrochemical groups. All main classes of polymers substance have been utilized in agricultural usage for the controlled release of pesticides (Akelah 2013). Cao et al. (2005) developed encapsulated acetamiprid diffusion-controlled microcapsules. Their diameter is ranging from 2 to 20 μm. Acetamiprid, an alkaline and high temperature-sensitive insecticide, was encapsulated using tapioca starch with urea and sodium borate as additives. The encapsulated acetamiprid reduced the degradation rate under heat about 60 days and UV radiation more than 48h, with no more than 3 % of degradation. This represents less than one tenth when compared to the UV degradation of commercial emulsifiable concentrate. Even in those conditions, it was also able to promote controlled liberation of the active compound for up to 10 weeks

depending on the formulation used. On the other hand, Bang et al. (2011) coated etofenprox and alpha-cypermethrin with chitosan. The coating layer becomes thicker in chitosan-coated nanoliposomes and the release period of etofenprox and alpha-cypermethrin in the core gets was longer with enhancing coating material doses and intrinsic surface charge. Add to this, Bhagat et al. (2013) used nanogel for the management of insect pests. He prepared this nanogel from the methyl eugenol with the help of low-molecular mass gelator, e.g., all-*trans* tri (*p*-phenylenevinylene) bis-aldoxime. This was proved to be very suitable for management of *Bactrocera dorsalis*, which is harmful pest of many fruit crops, including guava (*Psidium guajava*). Also, pyrifluquinazon nano-formulations loaded in the chitosan were very effective controlled-release features. It showed good potency after 14 days of treatment, while its strong lethal efficiency appeared after 2 days at 50 and 25 mg L^{-1}. NPs made of a chitosan nano-carrier and a metabolite from fungi *Nomuraea rileyi* showed more effectiveness against *S. litura* than the uncoated fungal metabolite and spores (Chandra et al. 2013). Pradhan et al. (2013) prepared polymeric acephate nanocapsule which was synthesized by polyethylene glycol. The obtained data suggested that the nanocapsule formulation had excellent activity against a broad spectrum of agriculturally harmful pests both in vitro and in vivo compared with the commercial formulation, and eco-friendly bio-compatible product.

Pant et al. (2014) carried out an experiment to enhance the eucalyptus oil (*Eucalyptus globulus*) activity as a pesticide. They used de-oiled cakes aqueous filtrate of jatropha (*Jatropha curcas*) and karanja (*Pongamia glabra*) extracting oil to prepare nanoemulsion for the management of *Tribolium castaneum*. The nanoemulsion with aqueous filtrate showed more toxicity (LC_{50} = 0.16 mg L^{-1}) and eucalyptus oil losses than the nanoemulsion without the aqueous filtrate (LC_{50} = 5.49 mg L^{-1}). Also, Forim et al. (2013) prepared Nano-/microparticles loaded with neem (*Azadirachta indica*)

extracts and used in controlling of *P. xylostella*. The bioassay results indicated that the efficacy of formulated products against *P. xylostella* showed 100% larval mortality. The nanoparticle information improved the stability of neem products against ultraviolet radiation and increased their dispersion in the aqueous phase. Add to this, Yang et al. (2009) reported that the polyethylene glycol (PEG) NPs along with garlic essential oil have toxic effects against store-product pests like *Tribolium castaneum*. Efficacy of NPs formulation were more than 80 % against the adult of *T. castaneum*, probably due to the persistent and slow release of components from NPs, whereas the efficacy of control treatments of free garlic essential oil was only 11 % at the same concentration (640 mg kg^{-1}). On the other hand, Saini et al. (2014) developed pyridalyl nanosuspension by using sodium alginate. They used this formulation as in vitro insecticidal form against *Helicoverpa armigera* larvae in comparison with commercial formulations. The micelle particle size of formulations was 138 nm. The bioassay results showed that nanoformulation was two and six folds more effective against *H. armigera* as compared with the commercial formulation and technical products.

Aouada et al. (2011) comprehensively described the use of biodegradable hydrogels as a potential delivery system for the controlled release of pesticides. For example, polyacrylamide–methylcellulose hydrogels presented high loading capacity of paraquat pesticide, up to 82 % of paraquat, in relation to the amount of paraquat available in the loading solution. It could be noted that the release of pesticides entrapped in a hydrogel occurs only after water penetrates the network to swell the polymer and dissolve the pesticides. Thus, the release of chemicals is closely related to the swelling characteristics of hydrogels, which, in turn, is a key function for the chemical architecture of hydrogels. In aforementioned paraquat preparations, pesticide diffusion capacity out of hydrogel was dependent on the swelling of the

matrix and the density of the network chains, that is, methylcellulose/acrylamide ratio and pore sizes, and the release of paraquat became slower when the methylcellulose and acrylamide concentrations increased. Also, Boehm et al. (2003) studied the ability of pesticide polymeric nanospheres formulation (NS) to improve the biodelivery of a new insecticide to plants, based on a nanoprecipitation method with Eudragit S100 polymer. Although the NS formulations are not as good as the classical suspension of ethiprole in terms of controlled release, the NS formulations enhance the systemicity of the active ingredient (a.i) and improve its penetration through the plant. Wang et al. (2017) prepared emamectin-benzoate (EB) slow-release microspheres by the microemulsion polymerization method. The results showed that the samples had uniform spherical shapes with an average diameter of 320.5 ± 5.24 nm, and good disparity in the optimal formulation with the polymeric stabilizer polyvinyl alcohol (PVA) and composite non-ionic surfactant polyoxyethylene castor oil (EL-40). The optimal EB pesticide slow-release microspheres had excellent anti-photolysis performance, stability, controlled release properties, and good leaf distribution.

2.2.2 Porous Hollow Silica Nanoparticles-Based Nanoformulations

Silica-based NPs are widely used in nanotechnology in the biomedical sector because they are easy to prepare and inexpensive to produce. Their specific surface characteristics, porosity, and capacity for functionalization make them good tools for biomolecule detection and separation, providing solid media for delivery systems and for agent protectors. They are used as safe for human organism and biocompatible with many active ingredients and additives (Ku et al. 2010; Bitar et al. 2012; Tang and Cheng 2013; Liu et al. 2014; Vaculikova et al. 2015). Silica-based NPs also found their application in agriculture, since silica materials can offer distinct advantages over other materials, as they provide more mechanically stable structures

than polymeric materials (Torney et al. 2007) and have structural flexibility in forming nanomaterials with high-capacity loading of active ingredients (Lou et al. 2008). Actually, A lot of work has been done on porous hollow silica nanoparticles in agricultural sector, for example Li et al. (2007) studied the efficiency of porous hollow silica nanoparticles (PHSN) as a carrier to improve the photostability of avermectin against UV radiation. The results demonstrated that the PHSNs carriers with a shell thickness of ~15 nm and a pore diameter of 4–5 nm have an encapsulation capacity of 625 g kg^{-1} for avermectin using a supercritical fluid loading method. The PHSNs carriers improved the photostability of avermectin by entrapping it into the hollow core of the nanoparticle carriers Lvov et al. (2008) developed halloysite aluminosilicate nanotubes with a 15 nm lumen, 50 nm external diameter and the length of 800 ± 300 nm as an entrapment system for loading, storage, and controlled release of anticorrosion agents and biocides. The halloysite tubes could be used as additives in biocide and protective coatings. Clay nanotubes (halloysites) were developed as carriers of pesticides with extended release and better contact with plants, enabling the reduction of the amount of pesticides by 70–80 %, which is then reflected in reduction of costs and the impact on water streams (Ditta 2012). Also, Popat et al. (2012) studied the efficacy of new insecticide formulation which was loaded imidacloprid into the mesoporous silica nanoparticles (MSNs) with different pore sizes, and the effect of pore size, surface area and mesoporous structure on uptake and release of imidacloprid. They found that the adsorption amount and release profile of imidacloprid were dependent on the type of mesoporous structure and surface area of particles. The controlled release nanoparticulate systems based on mesoporous silica nanoparticles (MSNs) were successfully used to adsorb and release imidacloprid and the MCM-48 mesoporous silica nanoparticles with a three dimensional (3D) open network structure showed the highest adsorption capacity

compared to other types of silica nanoparticles. Wibowo et al. (2014) reported that the sustained release of fipronil from oil-core silica-shell nanocapsules in vitro was tunable through the control of the silica-shell thickness (i.e., 8−44 nm), and the insecticidal effect of the fipronil-encapsulated silica nanocapsules against economically important subterranean termites could be controlled by tuning the shell thickness. Wang et al. (2014) prepared a novel Abamectin nanoformulation depended on porous silica nanoparticles as a delivery system to improve the chemical stability, dispersity, and the controlled release of Abamectin. They found that the nanoformulation can significantly improve the performance of controllable release, photostability, and water solubility of Abamectin by changing the porous structure of silica nanoparticles, which is promising to develop the efficacy and reduce the residues of pesticides.

Meyer et al. (2015) reported that the Functional Nano-Dispensers (FNDs), based on polymeric encapsulation of imidacloprid showed the FNDs were an effective releasing material for insecticides as compared with a standard commercial formulation, providing an acceptable level of protection for at least 10 days. Additionally, the FNDs could reduce the amount of imidacloprid required to cause mortality of *Diaphorina citri* as compared with the commercial formulation, while keeping similar efficacy. Moreover, FNDs have greater potential as a cost-effective solution against several pests. Guo et al. (2015) prepared a novel enzyme-responsive emamectin benzoate microcapsule by silica cross-linked with carboxymethylcellulose using epichlorohydrin. The results showed that the obtained microcapsules had a remarkable loading ability for emamectin benzoate and improved the photo- and thermal stability. The silica–epichlorohydrin–carboxymethylcellulose microcapsules displayed excellent cellulose stimuli-responsive properties and a sustained

insecticidal efficacy against *Myzus persicae* with less genotoxicity on *Allium cepa* chromosome compared with technical grade of emamectin benzoate.

CHAPTER 3.

Entomotoxic Effect of Silicon Dioxide Nanoparticles on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) Under Laboratory Conditions

ABSTRACT

The use of higher dosage and repeated applications of conventional pesticides have led to the rapid development of insect resistance to pesticide and adverse effects on human health and environment. Accordingly, researchers are prompted to identify an alternative entomologic agent for crop protection. Nanocides are being considered as alternatives to conventional insecticides because they are expected to lessen the application rate and reduce the chances of resistance development in pests. In this study, we evaluated the entomologic effects of nanosilica on larvae of *Plutella xylostella*, in a laboratory by using dust spray, larva dipping, leaf dipping, and solution spray methods. Dust treatment showed a more highly significant effect than the other three treatments. The mortality percentage increased up to 58 % and 85 % at 24 and 72 h after treatment, respectively, when nanosilica was applied at a rate of 1 mg cm^{-2}. In all four bioassays, mortality rate increased with both increased time after nanosilica exposure and increased concentration. Light microscopy and scanning electron microscopy images showed that larval death was due to desiccation, body wall abrasion, and spiracle blockage. These results suggested that nanosilica can be an alternative to conventional pesticides if dust formulation would be properly used.

Key words: Silicon dioxide, nanoparticles, nanosilica, *Plutella xylostella*, entomotoxicity.

3.1 Introduction

Pesticides are extensively used in agriculture to control insect pests and thus produce abundant food supply for a growing population. However, the indiscriminate use of pesticides has led to several problems, including environmental pollution, serious health hazards to humans and animals, pest resistance to pesticide, and destruction of beneficial insects. Owing to these environmental concerns, efforts have been raised to minimize pesticide environmental risks through the development of new and modern approaches for insect pest management.

Nanotechnology, which has been growing rapidly worldwide for the past decades, shows considerable promise for use in crop and foodstuff protection. Nanomaterials (NMs), or nanoparticles (NPs), are among the four broad areas (nanoelectronics, nanoinstruments, nanobiosystems, and nanoengineered materials) of nanotechnology. NPs are being preferentially harnessed because they offer a higher surface area and circulate more easily. In the past decade, NMs have provided a wide range of novel pesticide formulations or pesticide metallic NPs, such as nanoemulsion, nanocapsule, nanosuspension, and metallic oxide NP, with higher efficacy on pest control and lesser harmful effects on the environment compared with the traditional pesticide. Stadler et al. (2010) reported for the first time that alumina NPs has the insecticidal effect on two stored grain species, *S. oryzae* and *R. dominica*. Goswami et al. (2010) evaluated the control effect on *S. oryzae* of the different kinds of NPs, namely, silica nanoparticles (SNPs), aluminum oxide nanoparticles (ANP), zinc oxide nanoparticles (ZNP), and titanium dioxide nanoparticles (TNP). They found that SNPS and ANP were considerably more effective than ZNP and TNP and that the different insecticidal effects exist among various surface functionalized spherical SNPs. More recently, the entomotoxic effect of SNPs on stored grain insects, such as *S. oryzae* (Debnath et al. 2011), *C. maculatus* (Rouhani et al. 2012; Arumugam et al. 2016),

and *Corcyra cephalonica* (Vani and Brindhaa 2013), was reported. Debnath et al. (2012b) also indicated that the amorphous nanosilica (SNPs) could effectively kill the *S. litura* larvae at a dosage of 0.5 mg cm^{-2}. Based on the results, Debnath et al. (2012b) suggested that amorphous nanosilica can be used as a novel insecticide by the agricultural sector.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive pests of cruciferous crops worldwide; particularly cabbage, broccoli, and cauliflower especially in Asian countries (Talekar and Shelton 1993; Sarfraz et al. 2006). The average management expenses for DBM has been estimated at 4–5 billion US$ (Zalucki et al. 2012). Also, it has short generation time, genetic plasticity, high fecundity, and particularly the intensive selection pressure (Jiang et al. 2015), it was the first insect pest reported to develop resistance against dichlorodiphenyltrichloroethane, shortly after three years of the chemical use (Angkersmit 1953). This insect also subsequently showed significant resistance to most insecticides, including recently introduced compounds with new modes of action, such as spinosad, avermectins, indoxacarb, the bio-pesticide *Bacillus thuringiensis* cry toxins, and the anthranilic diamide chlorantraniliprole (Li et al. 2006; Sayyed and Wright 2006; Zhao et al. 2006; Pu et al. 2010; Troczka et al. 2012; Wang and Wu 2012; Zhen-di et al. 2012; Sukonthabhirom and Siripontangmun 2013; Wang et al. 2013). Many reports of control failure of this pest are often documented. In this study, our main objective is to test the insecticidal properties of SNPs as an alternative to conventional pesticides against *P. xylostella*, in addition, to find the best application method for nanosilica to control this pest. In this study, our main objective is to test the insecticidal properties of SNPs as an alternative to conventional pesticides against *P. xylostella*, in addition, to find the best application method for nanosilica to control this pest.

3.2 Materials and methods

3.2.1 Preparation of silicon dioxide NPs

Nanosilica was prepared using a sol–gel technique according to Musić et al. (2011) method with some modifications. A total of 0.2 g equivalent sodium silicate (Na_2SiO_3) (Sigma-Aldrich, St. Louis, MO, USA) was diluted in 300 mL ultrapure water, and 0.2 g equivalent sulfuric acid (H_2SO_4) (Sigma-Aldrich) was diluted in 200 mL ultrapure water. The H_2SO_4 solution was added slowly drop by drop into the sodium silicate solution. The mixture was mixed with a magnetic stirrer for 45 min to form a nanosilica gel. The gel was washed six times in a filter paper with ultrapure water to remove sodium sulfate (Na_2SO_4) from the mixture under vacuum filtration. The gel was freeze-dried (ALPHA1-2 LD plus, Martin Christ Gefriertrocknungsanlagen, Osterode, Germany) for 24 h and finally calcined at a temperature of 500 °C for 2 h to obtain Sio_2 NPs.

3.2.2 Characterization of SNPs

The surface morphology and average particle size of nanosilica were measured using field emission scanning electron microscope (SU8010 UHR FE-SEM, Hitachi, and Tokyo, Japan) and transmission electron microscope (Tecnai^{TM} Spirit TEM, FEI Company, Hillsboro, OR, USA). The powder sample X-ray diffraction analysis was performed using a multipurpose X-ray diffractometer (XRD, X'Pert PRO, PANalytical, Almelo, The Netherlands) with Cu radiation (λ = 1.54 A) at 40 kV and 40 mA. 121 The samples were scanned from 3 to 30 °θ, and the scanning rate was 2 °θ per min with step size of 0.02 °θ.

3.2.3 Insect culture

The initial population of *P. xylostella* was collected from a cruciferous vegetable field in the eastern suburb of Hangzhou (30 14’ N, 120 15' E), Zhejiang Province, China, in September 2014. *P. xylostella* was reared at 25 ± 2 °C with 70 % ± 10 % relative humidity (RH) and a light–dark cycle of 16:8 h. The larvae were reared on cabbage leaves (*Brassica oleracea* var. capitata (L.) (Capparales: Brassicaceae) cv. Jingfeng No. 1), and adults were fed with 10 % sucrose solution (Gu et al. 2015).

3.2.4 Bioassay experiments

The bioassays were performed in plastic containers (14.2 X 7.2 X 5.2 cm) using dust spray, leaf dipping, larva dipping, and solution spray methods. All bioassays were carried out at 25 ± 2 °C with 70 % ± 10 % RH and a light–dark cycle of 16:8 h. The early third instar larvae were used in all experiments. Three replicates for each concentration were performed, and 30 larvae were utilized for each replicate. Insect mortality was recorded at24, 48, and 72 h after the larvae were exposed to nanosilica.

3.2.4.1 Dust spray

For the dust spray bioassay, five application rates at 0.125, 0.25, 0.5, 0.75 and 1 mg cm^{-2} were used for the experiment. These rates were chosen according to previous study Debnath (et al. 2012a). Thirty early third instar larvae were placed in a plastic cup and then exposed to nanosilica powder through a mini air compressor AS18BK with airbrush HS-178 under pressure 2 kg cm^{-2} (Haosheng Pneumatic Machinery, Ningbo, China). After exposure, the larvae with nanosilica powder were reared in a clean plastic container with one leaf disc (as food) (2.5 cm in diameter) of fresh cabbage without nanosilica dust, then the containers covered by a perforated cover for aeration. The leaf discs were replaced every day with fresh and clean one. The mortality was recorded 24, 48, and 72 h after exposure to nanosilica. The death was

judged from the larval response to gentle prodding with a small Chinese writing brush, and the dead larvae were checked under a light microscope (LEICA MZ 95 microscope equipped with LECIA DFC 300 FX camera, LEICA, Wetzlar, Germany) and tabletop microscope (Tabletop Microscope TM-1000, Hitachi,) to investigate the causes of the larvae's death. The larvae that were not exposed to nanosilica powder served as control.

3.2.4.2 Leaf dipping

Nanosilica solutions with different concentrations were prepared using ultrapure water, the concentrations were 100, 200, 400, 800 and 1200 mg L^{-1} and the total volume was 100 mL for each concentration. Leaf dipping bioassay was utilized to test the stomach toxicity of the nanosilica to the DBM larvae. Cabbage leaf was cut into discs (2.5 cm in diameter) and dipped into the test solution for 30 s with gentle agitation. Those leaf discs dipped in ultrapure water served as control. Thirty minutes later, the surface of leaf discs was dried through air dry, one leaf disc with 30 early third instar larvae were placed in a plastic container with a perforated cover for aeration. The leaf disc was replaced every day with a fresh and clean one.

3.2.4.3 Larva dipping

Larva dipping was performed to study the contact toxicity of the nanosilica to DBM larvae. The early third instar larvae were immersed into prepared nanosilica solution for 10 s, the concentrations were 100, 200, 400, 800, and 1200 mg L^{-1} and the total volume was 100 mL for each concentration. Those larvae immersed in ultrapure water served as control and all larvae were reared in a plastic container with fresh cabbage leaf discs (2.5 cm diameter) without any nanosilica and covered by a perforated cover for aeration. The leaf discs were replaced every day with fresh and clean ones.

3.2.4.4 Solution spray

Thirty early third instar larvae were induced into a plastic container and sprayed by a mini air compressor AS18BK with airbrush HS-30 K (Haosheng Pneumatic Machinery) under pressure 2 kg cm^2 with different concentrations of nanosilica, the concentrations were 100, 200, 400, 800, and 1200 mg L^{-1}, each replicate (container) was sprayed with 2 mL nanosilica solution. The control larvae were sprayed with 2 mL ultrapure water. After spray, the larvae were immediately transferred into a clean plastic container and reared with one fresh cabbage leaf disc (2.5 cm in diameter) without any nanosilica and covered by a perforated cover for aeration. The leaf disc was replaced every day with fresh and clean one.

3.2.5 Statistical analysis

The mortality was analyzed via a two-way analysis of variance (ANOVA) with IBM SPSS Statistics 19 software. All percentage data were transformed using arcsine square root before ANOVA to standardize means and normalize variances and were transformed back to percentages for presentation. Mean values were separated through the least significant difference (LSD) test ($P < 0.05$) when significant differences among several mean values were detected by ANOVA. In terms of dust spray bioassay, the LC_{50} values were calculated using the statistical method of LdP line program software (http://embakr.tripod.com/ldpline/ldpline.htm), which was devoted to the calculation of probit analysis based on Finney's (1971) method .

3.3 Results

3.3.1 NP characterization

FE-SEM and TEM images confirmed that the silica particles had nanometric sizes (Figure 1). The nanosilica particles had spherical shape with a small percentage of irregular surfaces, and the particle size ranged from 17 to 38 nm with an average size

of 25 nm (Figure 1). Figure 2 shows the XRD pattern of nanosilica, and the peak was observed at 21.218 °θ using Bragg's law, that is, λ = 2dSin θ. The results show a single broad peak for an amorphous nanosilica core region. Therefore, this makes the SNPs suitable for biological application.

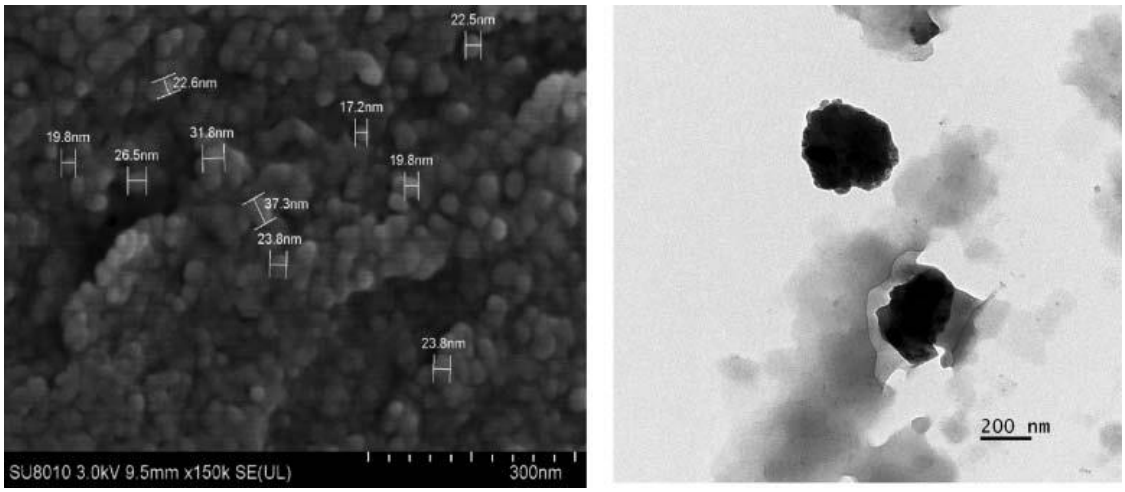

Figure 1: Images of nanosilica (left: FE-SEM, right: TEM). The nanosilica particles had spherical shape with a small percentage of irregular surfaces

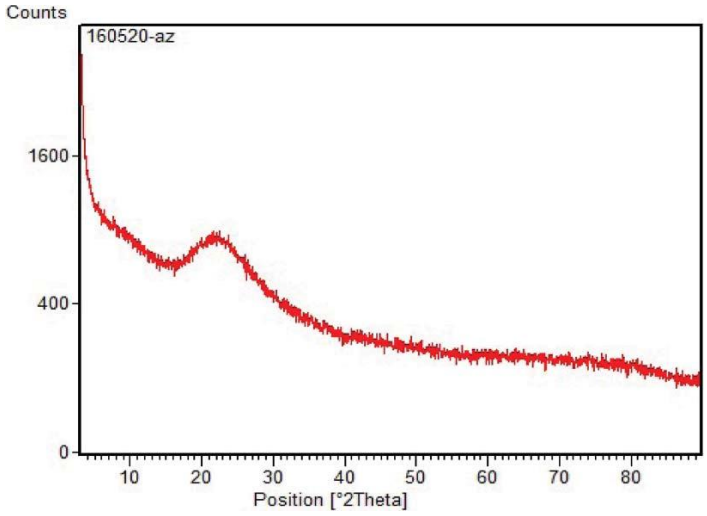

Figure 2: XRD pattern of nanosilica show a single broad peak for an amorphous nanosilica core region.

3.3.2 Bioassay results

When two factors (time and concentration) were considered separately, all treatment effects were significant. But when interactions of two factors were considered, dust treatment was significant and other three treatments were not significant at $P > 0.01$ level. The dust bioassay produced the highest mortality among all four bioassays. The higher the concentration applied, the higher the mortality was at the same recording time (Table 1). Regarding the time interval after dust spray, the longer the interval was, the higher the mortality was. Nanosilica dust could kill more than 80 % larvae when applied at 1 mg cm^{-2} (Table 1). The LC_{50}, LC_{90} and their confidence limits of nanosilica against *P. xylostella* were calculated (Table 2). The LC_{50} values ranged from 0.99 to 0.36 mg cm^{-2}, while the LC_{90} values ranged from 4.95 to 1.49 mg cm^{-2} at 24–72 h after spray. Both values sharply decreased with the increase in time duration, suggesting that toxicity sharply increased with the passage of time. The images obtained using light microscope showed that the larval corpse was extremely dry, while those from tabletop microscope illustrated that parts of the larval cuticle and legs were damaged and covered by nanosilica (Figure 3 and 4). Both leaf and larva dipping could kill some larvae, but they did not produce a higher mortality (Table 3) under all test concentrations. The highest mortality was found 72 h after immersion at an applied rate of 1200 mg L^{-1} through larva dipping, but the mortality rate was still substantially lower than we expected. The nanosilica solution spray could also kill some larvae but also did not produce higher mortality (Table 3). The highest larvae mortality, which amounted up to nearly 40 %, was found at 72 h after exposure when nanosilica was applied at 1200 mg L^{-1}.

Table 1. Mortality (mean ± SD %) of the third instar larvae of *P. xylostella* after SNPS exposure via dust spray.

Conc. mg cm^{-2}	Mortality		
	24 h	48 h	72 h
0.125	7.8 ± 1.9 e	11.1 ± 1.9 e	18.9 ± 1.9 e
0.25	12.2 ± 1.9 d	20.0 ± 3.3 d	33.3 ± 3.3 d
0.5	22.2 ± 1.9 c	38.9 ± 3.9 c	56.7 ± 3.3 c
0.75	38. 9 ± 3.9 b	58. 9 ± 5.1 b	75.6 ± 5.1 b
1.00	57. 8 ± 1.9 a	70.0 ± 3.3 a	84.4 ± 3.9 a
control	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f

Note: Means within a column followed by the same letters are not significantly different at P > 0.05 (Fisher LSD test). Mean is calculated from three repetitions, each has 30 larvae tested.

Table 2. $LC_{50\text{-}90}$ values of SNPs against the third instar larvae of *P. xylostella* via dust spray.

Treatments	Time	LC_{50} (mg cm^{-2}) (LCL–UCL)	LC_{90} (mg cm^{-2}) (LCL–UCL)	Slope ± SE	x^2
Dust	24 h	0.99(0.81–1.29)	4.95(3.12–10.42)	1.83±0.22	6.77
	48 h	0.60(0.52–0.70)	2.63(1.91–4.17)	1.99±0.20	2.46
	72 h	0.36(0.32–0.41)	1.49(1.18–2.05)	2.10±0.19	2.20

Note: LCL: lower confidence limit and ULC: upper confidence limit.

Although the mortality was substantially higher compared with those in leaf and larva dipping bioassays, it was still considerably lower than we expected.

Figure 3: Light microscope images of dead larvae *Plutella xylostella* after nanosilica treatment. The larvae were extremely dry after nanosilica dust treatments.

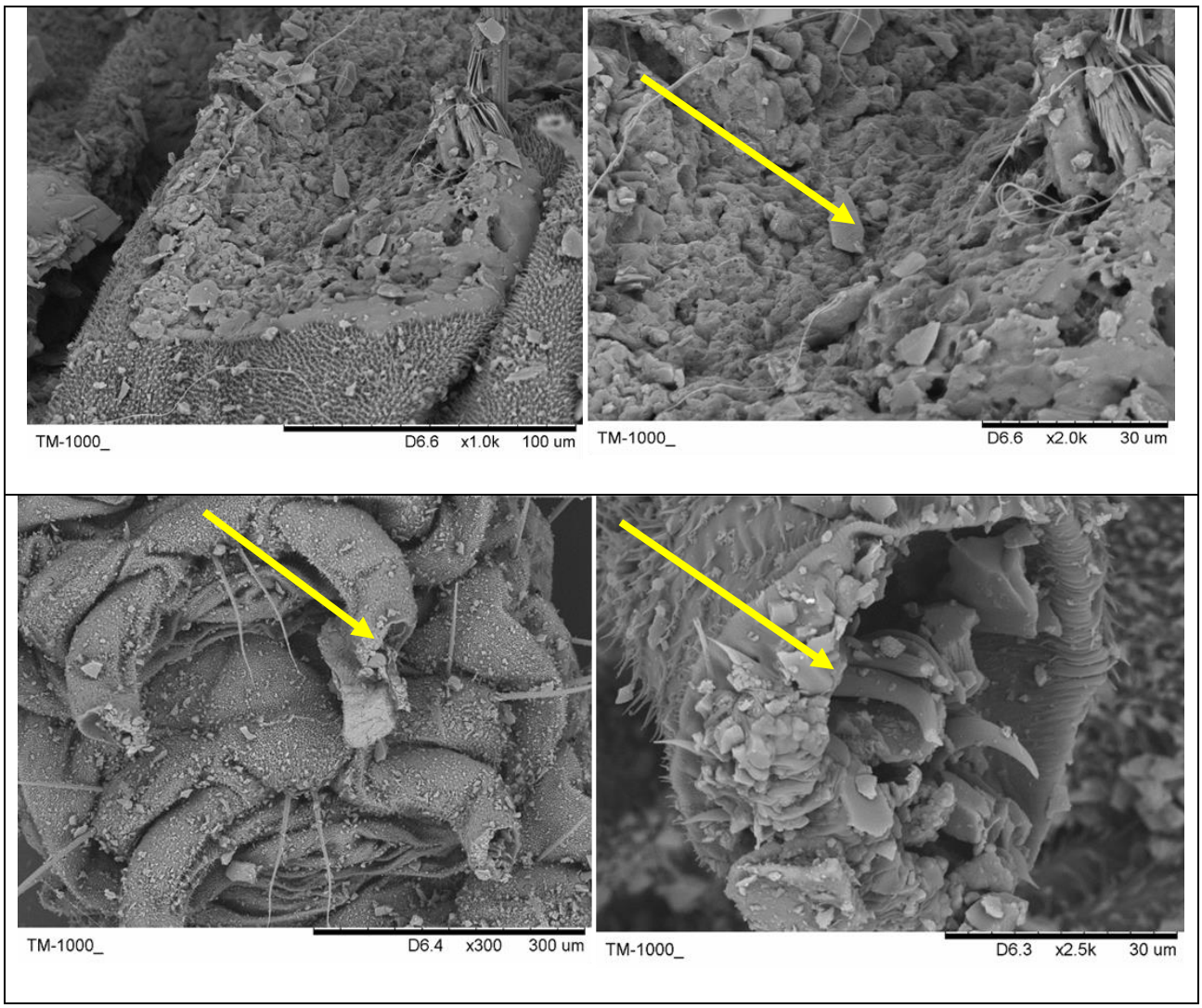

Figure 4: Tabletop microscope TM-1000 images after nanosilica treatment (up-left: some portions of damaged insect cuticle, up-right: the amplification of the right image, down-left: damaged insect legs, down-right: the amplification of the part arrowed).

Table 3. Mortality (mean ± SD %) of the third instar larvae of *P. xylostella* after SNPs exposure via leaf dipping, larva dipping, and solution spray.

Treatment	Concentration (mg L^{-1})	hours after treatment		
		24	48	72
leaf dipping	100	4.4±1.9 d	6.7±0.0 d	8.9±1.9 b
	200	6.7±0.0 cd	7.8±1.9 cd	11.1±1.9 b
	400	7.8±1.9 bc	11.1±1.9 bc	16.7±0.0 a
	800	10.0±0.0 ab	14.5±3.9 ab	18.9±5.1 a
	1200	12.2±1.9 a	15.6±3.9 a	20.0±3.3 a
	control	0.0±0.0 e	0.0±0.0 e	0.0±0.0 e
Larva dipping	100	6.7±3.3 b	8.9±5.1 b	14.4±1.9 c
	200	7.8±1.9 ab	11.1±5.1 bc	17.8±1.9 bc
	400	8.9±1.9 ab	12.2±5.1 ab	20.0±3.3 ab
	800	11.1±1.9 ab	16.7±3.3 ab	22.2±5.1 ab
	1200	13.3±3.3 a	17.8±5.1 a	23.3±3.3 a
	control	0.0±0.0 e	0.0±0.0 e	0.0±0.0 e
solution spray	100	7.8±1.9 d	10.0±3.3 d	15.6±5.1 d
	200	8.9±1.9 cd	13.3±3.3 cd	18.9±3.9 cd
	400	11.1±1.9 bc	15.6±1.9 c	23.3±3.3 bc
	800	12.2±1.9 ab	21.1±1.9 b	28.9±5.1 b
	1200	14.4±1.9 a	26.7±3.3 a	37.8±1.9 a
	control	0.0±0.0 e	0.0±0.0 e	0.0±0.0 e

Note: Means within a column under the same treatment followed by the same letters are not significantly different at $P > 0.05$ (Fisher LSD test). Mean is calculated from three repetitions, each has 30 larvae tested.

3.4 Discussion

The shape and size of the nanosilica prepared in this study were checked using SEM and TEM. The SEM and TEM images showed that the nanosilica has an amorphous spherical morphology with diameter varying between 17 and 38 nm. This size is similar to the size of the nanosilica prepared by Rouhani et al. (2012). The powder XRD pattern obtained showed a single broad peak at 21.218 °θ, which also indicates its amorphous nature. This result was in good agreement with the earlier reported

findings (Debnath et al. 2012b; Vani and Brindhaa 2013). Both studies reported that the peak was approximately at 20 °θ.

The amorphous micron sized silica, has become most popular (Athanassiou et al. 2007, 2008) as an alternative to the conventional pesticides which have mammalian toxicity. The use of nanomaterials in agriculture is still at a rudimentary stage. Our study demonstrated that SNPs could kill the larvae of *P. xylostella*. This result is consistent with earlier reports on other field pests, such as *Lipaphis pseudobrassicae* (Goswami et al. 2010) and *S. litura* (Goswami et al. 2010; Debnath et al. 2012b). Goswami et al. (2010) reported that the amorphous nanosilica showed high efficiency against two stored grain pests *S. oryzae, Tribolium castaneum* and two field pests *Lipaphis pseudobrassicae* and *S. litura* as a potent and safe pesticide. Debnath et al. (2012b) showed that the amorphous SNPs could effectively kill the *S. litura* larvae at a dosage of 0.5 mg cm^{-1}. Based on the results, they suggested that amorphous nanosilica can be used as a novel insecticide by the agricultural sector. In addition, Rouhani et al. (2012) reported that ZnO –TiO_2 – Ag NPs exhibited insecticidal activity *Frankliniella occidentalis* (Pergande). They also showed that most mortality effects occurred under 28 % ZnO–70 % TiO_2–2 % Ag (LC_{50} = 195.27 mg L^{-1}). The mortality is substantially higher when SNPs was applied in dust than when SNPs was used as a solution. The enhanced mortality rate under dust treatments might be caused by the absorption of water through the disruption of cuticle by nanosilica, which ultimately results to insect death via desiccation. On the contrary, when nanosilica was applied in the form of water solution, the water absorption function of SNPs from the larval cuticle was lost prior to application. Thus, SNPs could no longer absorb water from the larval cuticle. In all the four bioassays, the mortality rate increased with the increase in SNPs exposure time and concentration. These results were in agreement with those of the previous studies not only on *S. oryzae* (L)

(Goswami et al. 2010; Debnath et al. 2011), *C. cephalonica* (S) (Vani and Brindhaa 2013), and *Callosobruchus Maculatus* F. (Rouhani et al. 2012; Arumugam et al. 2016) in stored grains but also on *S. litura* in field crop (Debnath et al. 2012b).

However, dust application of the nanosilica was not as effective as that reported by Debnath et al. (2012b). They reported that 0.125 mg cm^{-2} hexamethyldisilazane and 3-mercaptopropyl trimethoxysilane capped SNPs could kill 58 % and 64 % of the second instar *S. litura* larvae, respectively. Moreover, no survivors were observed at 24 h after treatment with both SNPs at 0.5 mg cm^{-2}. Two reasons might contribute to the difference between our and their results. One reason is that our dust was not capped with any additional groups, such as thiol and methyl. The other one is that different insect species were used in the two experiments. The second instar larvae of *S. litura* may be considerably more sensitive to desiccation than the third instar larvae of *P. xylostella*. Furthermore, the thickness and structure of their cuticle might be different.

Barik et al. (2008) have reviewed that the nanosilica (~3–5 nm) could be successfully used to control a range of agricultural insect pests and animal ectoparasites of veterinary importance. The control mechanism of insect pest by using nanosilica is depend on the different insect pests used cuticular lipids for protecting their water barrier and thereby prevent death from desiccation. But nanosilica absorbed in these cuticular lipids by physisorption means and thereby (when applied on leaves and stem surface) causes death of insects purely by physical means (De et al. 2014). Our study confirmed that superior entomotoxicity of dust is caused by SNPs absorption of water from the larval cuticles. The larvae begin to lose water through desiccation as the water barrier is damaged and finally die owing to desiccation, leaving their hard corpses behind. Nanosilica dust also induced abrasion on insect cuticle which enhanced the larval mortality (Debnath et al. 2012b). After solution spray, the water

on SNPs surface was reduced, and abrasion owing to SNPs was enhanced as the exposure time increased. As a consequence, the number of larval death increased. This finding is supported by our bio- assay results (Table 3). Spiracle blockage of the larvae treated with SNPs was also observed. This might be another reason for the larval death. The attached dust around the spiracle absorbed water, causing larger volume, and finally lead to the occurrence of blockage and damage. The death caused by desiccation, abrasion, and blockage was reported by several earlier researchers (Debnath et al. 2011; Debnath et al. 2012b; Rouhani et al. 2012; Vani and Brindhaa 2013; Arumugam et al. 2016;). These physical entomocidal modes of action will make SNPs more effective in the control of insect pests (Vani and Brindhaa 2013; Arumugam et al. 2016).

Many previous studies have confirmed that NPs, whether metal or nonmetal, can be used to control plant and animal pathogens and protect the economic crops and stored grains from insect attack (Barik et al. 2008; Gajbhiye et al. 2009; Goswami et al. 2010; Debnath et al. 2011; Rouhani et al. 2012; Arumugam et al. 2016). The data obtained from this study warrant further research to explore the potential of SNPs to control pests on field crop. However, alumina NPs present on the groundwater inhibits the growth of some plants, including carrot, cabbage, cucumber, corn, and soybean. SNPs has no adverse effect on plant growth; rather, silica enhances the structural rigidity and strength of plants (Ebeling 1971; Epstein 1994). When used as a seed protectant, SNPs has no any adverse effect on the germination and growth of pulse seeds treated at 500 and 1000 mg L^{-1} concentrations, and no alteration was observed in the seed coat upon SNPs treatment (Arumugam et al. 2016). This may be one of the possible reasons for the age-old tradition of using silica dust as a protective agent for stored seed by different ethnic races all over the world (Ebeling and Wagner

1959; Ebeling 1971). Amorphous silica has also been declared safe by the US Food and Drug Administration (Goswami et al. 2010; Stathers et al. 2004).

Silicon can be taken up by plants in the form of monosilicic acid ($Si(OH)_4$) and transported from the root to the shoot, enhancing plant constitutive defenses against abiotic and biotic stresses, inducing defenses of plants attacked by fungal pathogens (Savvas et al. 2009) and arthropod pests (Gomes et al. 2008), attracting more natural enemies by triggering production of herbivore-induced plant volatiles (Kvedaras et al. 2010). Considering that amorphous silica is relatively biosafe, SNPs-based nanocides can be an alternative to the conventional hazardous insecticides. Moreover, insects are very unlikely to build up resistance against these physically active nanocides. However, the use of NMs in agriculture is still at a rudimentary stage, and it will be premature to comment on its toxicity in living system (Debnath et al. 2011). Aside from the practical use of SNPs on the control of insect pests in agriculture, further research is necessary to address the safety issues of nanosilica on the beneficial insects and human health, for example, the inhalation of the dust leading to silicosis of the respiratory system. In addition, more studies need to be conducted regarding the removal of residues on the surface of agricultural products upon dust application and the effects of accumulation nanosilica on the environmental after application on the field. The development of nanosilica formulations to improve potency while reducing the dosage through improvements in the delivery system via NM encapsulation as well as to decrease the cost also need to be explored. Although insects are unlikely to become genetically selected or physiologically resistant to such mechanism of action, the concern that insect may develop a behavioral response to these particles and thus avoid contact also needed to be investigated.

CHAPTER 4.

Preparation and Characterization of Emamectin Benzoate Nanoformulations Based on Colloidal Delivery Systems and Use in Controlling *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

Abstract

Colloidal delivery systems have been widely used as carriers for controlled delivery of pesticides to improve the efficacy and photostability of natural and semi-synthetic pesticides. In this study, we have synthesized emamectin benzoate nanoformulations (EB+NFs) depending on polymeric nanocapsules (PNC) and two types of the nanosilica, mesoporous nanosilica (MCM-48) and silicon dioxide nanoparticles (SNPs) as carriers for the emamectin benzoate (EB). The fabricated nanoformulations were characterized by using X-ray diffraction analysis, Fourier transform infrared spectroscopy, particle size, zeta potential, morphology, absolute recovery (AR), entrapment efficiency (EE), UV stability and release kinetics. The obtained results showed that the carriers had a remarkable loading ability for EB and improved the EB photostability. The EE % of nanoformulations were 92.84 %, 87.45 % and 71.19 % for emamectin benzoate polymeric nanocapsules (EB+PNC), emamectin benzoate SNPs (EB+SNPs) and emamectin benzoate MCM-48 (EB+MCM-48) respectively. The insecticidal activity of (EB+NFs) against *Plutella xylostella* showed that the (EB+SNPs) was more effective than other EB+NFs and EB alone. The LC_{50} values were 0.18, 4.03, 8.49 and 11.06 mg L^{-1} for EB+SNPs, EB+MCM-48, EB+PNC and EB respectively. The obtained results suggest the colloidal delivery systems that used in this study could improve the efficacy and photostability for EB, and they are able to overcome the disadvantage of the natural and semi-synthetic pesticides such as

environmental sensitivity and to increase the efficacy of pesticides, which eventually leads to reduce the dosage of pesticides needed, reducing the number of applications required in comparison to conventional formulations.

Key words: Emamectin benzoate, nanocapsules, colloidal delivery systems, *Plutella xylostella*.

4.1 Introduction

Herbivorous insects are very destructive pests for the important crops and their production. These insects cause damage by feeding on seedlings, germinating seeds and flowers. The chemical pesticides are useful for protecting the crops from insect damage during the growing season. However, indiscriminate use of them has led to several environmental problems, including serious health hazards to humans and animals, development of insecticide resistance, destruction of beneficial insects and accumulation of pesticide residues in different environmental compartments.

In the recent years, natural and semi-synthetic pesticides have gained interest as a promising alternative to conventional pesticides for pest insect control (Forim et al. 2013), but these pesticides exist obviously some disadvantages, including low activity and short persistent under various environmental conditions such as sunlight, humidity and rainfall. Emamectin benzoate (EB) is a semi-synthetic derivative of abamectin of the avermectin family of 16-membered macrocylic lactones. This epi-methyl amino derivative showed increased effectiveness against a broad spectrum of lepidopterous and coleopterous pests with application rates in active ingredient (a.i.) ranging between 8.4–16.8 g ha^{-1} (Jansson et al. 1997; Krämer and Schirmer 2007).

Unfortunately, the avermectin compounds are degraded rapidly from the environment after application. The binding compounds to the soil are rapidly decomposed by the soil microorganisms after fast photolysis on plant surfaces. Additionally, the current

commercial formulations of EB are sensitive to the light and temperature. These problems limit the use of EB in agriculture sector because an insecticide should persist in the field for enough time to ensure adequate control of pests (Krämer and Schirmer 2007). Therefore, there is need to develop formulation that could meet the requirements of high efficiency and prolonged protection.

Colloidal delivery systems such as polymeric nanocapsules (PNC) and polymeric nanospheres (PNS) have been used to overcome these disadvantages and to improve the insecticidal properties according to the principle of controlled release formulations (CRFs). CRFs have the ability to reduce the environmental hazards of excessive use of pesticides around the world to protect crops. In many cases the use of CRFs could reduce the total applied amount of pesticide a.i. by reducing the concentration and time of application leading to reduce economic cost as compared with conventional formulations. Consequently, it could lower its residue on agricultural products and the risks to humans and the environment (Akelah 1996; Cao et al. 2005; Akelah 2013).

Colloidal delivery systems show high efficiency as a means of efficiently delivering one or a mixture of active ingredients to their site of action. Furthermore, PNC can reduce the side effects of the insecticides and improve the photostability of active ingredients (Charcosset et al. 2005). A lot of work has been done on colloidal delivery systems in agricultural sector. Acephate polymeric nanocapsule synthesized with polyethylene glycol (PEG-400) showed increasing solubility in water, increasing stability and efficiency at a lower dose, reducing the economic cost for each application, and decreasing acephate toxicity to beneficial insects when compared with the commercial acephate formulation (Choudhury et al. 2012; Pradhan et al. 2013). Polyethylene glycol (PEG) coated nanoparticles (NPs) loaded with garlic essential oil were efficient against adult *Tribolism castaneum*. The control efficiency

against adult *T. castaneum* was remained over 80 % after five months with NPs loaded garlic essential oil but was only 11 % with garlic essential oil alone (Yang et al. 2009). The EB microcapsules based on a copolymer matrix of silica–epichlorohydrin–carboxymethylcellulose could protect effectively EB against photo- and thermal degradation and thereby increase their efficacy against *Myzus persicae*. (Guo et al. 2015).

The mesoporous silica nanoparticulates (MSNs) can increase the stability, dispersity, and the controlled release of pesticide compounds under environmental condition. The photostability of avermectin was improved by the porous hollow silica nanoparticle (PHSNs) carriers entrapping it into the hollow core of the nanoparticle carriers (Li et al. 2007). Also the PHSNs improved the efficacy of controllable release, photo-stability and water solubility of abamectin by modifying the porous structure of silica nanoparticles, which is useful to enhance the bioavailability and decrease the residues of pesticides (Wang et al. 2014). In another study, it was found that the CRFs based on MSNs were successfully used to adsorb and release imidacloprid (Popat et al. 2012). Also, polymeric encapsulation based Functional Nano-Dispensers (FNDs) of imidacloprid showed that the FNDs were an effective releasing material for insecticides as compared with a standard commercial formulation, providing an acceptable level of protection for at least 10 days. Additionally, the FNDs could reduce the amount of imidacloprid required to cause similar mortality of *Diaphorina citri* as compared with the commercial formulation. Moreover, FNDs have greater potential as a cost-effective solution against a number of pests (Meyer et al. 2015).

In this work, three novel functionalized EB+NFs emamectin benzoate polymeric nanocapsules (EB+PNC), emamectin benzoate silicon dioxide nanoparticles (EB+SNPs) and emamectin benzoate mesoporous nanosilica (EB+MCM-48), were

prepared based on colloidal delivery systems to improve the EB stability under various environmental conditions such as sunlight and humidity. The EB+NFs were characterized regarding their particle size distribution, zeta potential (ZP), entrapment efficiency (EE), and morphology. Photostability studies were also performed for all EB+NFs *in vitro* condition. The toxicity of three EB+NFs against diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), were evaluated and compared with EB alone.

4.2 Materials and methods

4.2.1 Reagents and Chemicals

Emamectin benzoate (70 %) was kindly provided by Institute of Pesticide & Environmental Toxicology, Zhejiang University. Ethyl cellulose, sodium silicate (Na_2SiO_3), sulfuric acid (H_2SO_4), cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS, 98 %) and ammonium hydroxide (NH_4OH) solution were purchased from Sangon Biotech (Shanghai) Co., Ltd, (Shanghai, China). All organic solvents (methanol, ethanol, acetonitrile, acetic acid, and dichloromethane) [high-performance liquid chromatography (HPLC) grade], Pluronic® F-127 (nonionic surfactant), sorbitan monostearate (Span 60) and sorbitan monooleate (Tween 80) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced in our laboratory using a Milli-Q System (18 MΩ) (Millipore Corp., Bedford, MA, USA).

4.2.2 Preparation of EB+NFs

4.2.2.1 Preparation of EB+PNC

The EB+PNC was prepared according to Forim's et al. (2013) method with some modifications. Nanoemulsion was prepared by vigorous homogenization. The mixture of 0.2 g a.i. of EB dissolved in 5 ml methanol and 0.2 g of Span 60 poured in

100 mL water was stirred through Ultra-Turrax homogenizer (IKA T 10 B S25 basic Ultra-Turrax; Ika-Werke, Germany) at 28000 g for 5 min to produce EB nanodrops. After a brief period of stabilization, the solution of 0.5 g of ethyl cellulose dissolved in 20 ml ethanol was poured into nanodrops of EB under magnetic stirring by mini air compressor AS18BK with airbrush HS-30K (Haosheng Pneumatic Machinery Co., Ltd, Ningbo, China), under pressure 30 psi. After stirring above mixture for 10 min, the third solution of 0.2 g of Tween 80 in 20 mL water was poured to the previously made solution under magnetic stirring for 10 min.

4.2.2.2 Preparation of EB+SNPs

The SNPs was prepared using sol–gel technique in accordance with the Musić's method (Musić et al. 2011) with some modifications. A total of 0.2 g equivalent sodium silicate (Na_2SiO_3) was diluted in 300 ml ultrapure water, and 0.2 g equivalent sulfuric acid (H_2SO_4) was diluted in 200 ml ultrapure water. The H_2SO_4 solution was added drop by drop into the sodium silicate solution. The mixture was mixed through a magnetic stirrer for 45 min to form a nanosilica gel. The gel was washed six times in a filter paper with ultrapure water to remove excessive sodium sulfate (Na_2SO_4) from the mixture under vacuum filtration. The gel was dried by using an ALPHA 1-2 LD plus freeze dry (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h to obtain SNPs powder.

The SNPs loaded with EB were prepared by a freeze-drying technique, colloidal solution from SNPs and EB (a.i.) were mixed at a ratio of 1:1 by weight. Firstly, 0.8 g SNPs was diluted in 200 ml ultrapure water, and the SNPs colloidal solution was sonicated for 30 min to ensure the nanoparticles are separated from each other. Then, 0.8 g EB (a.i.) was dissolved in 20 ml methanol. The EB solution was added drop by drop into the colloidal SNPs under continuously stirred condition by the magnetic stirrer at speed 600 rpm for 2 h at room temperature. After 2 h the EB could be

completely lodged on the surface of SNPs and then the mixture was dried by using the ALPHA 1-2 LD plus freeze dry for 24 h to get EB+SNPs powder.

4.2.2.3 Preparation of EB+MCM-48

The MCM-48 nanoparticles were prepared using the method of Kim's et al. (2010). Briefly, 0.5 g CTAB and 2.05 g Pluronic F127 were dissolved in a solution of ultrapure water (96 mL), ethanol (34 ml) and 29 % (by weight) ammonium hydroxide solution (10.05 ml) at room temperature. After complete dissolution, 1.8 g of TEOS was added into the mixture at once. After 1 min of mechanical stirring at 1000 rpm, the mixture was kept at a static condition for 24 h to generate silica nanoparticles. Resulting white precipitates were collected, centrifuged and washed twice with ethanol, and dried under vacuum. Finally, the dried precipitates were calcined at 550 °C for 5 h.

Loading experiments were carried out in methanol as EB is highly soluble in methanol. EB (a.i.) and the MCM-48 carriers were mixed at a weight ratio of 2:1 (a.i. EB: MCM-48). The MCM-48 (0.5 g) were suspended in 100 mL methanol and sonicated for 30 min. EB (1 g a.i.) was dissolved in 50 mL methanol, then the EB solution was added drop by drop into MCM-48 suspensions. Whole solution was continuously stirred by magnetic stirrer at 600 rpm for 30 min at room temperature. The mixed solution was shaken for 24 h, a time period found to be sufficient to reach equilibrium. After 24 h impregnation, the suspensions were used to evaluate EB+MCM-48 (Popat et al. 2012).

4.2.3 Determination of emamectin benzoate content

4.2.3.1 Determination of emamectin benzoate in EB+PNC

The total amount percentage of absolute recovery (AR %) of EB in EB+PNC was determined using the following method. First, 0.1 mL of the EB+PNC was dissolved

in 0.9 mL of ethanol for two hours. After polymer dissolution, the solution was centrifuged (centrifuge 5417 R; Eppendorf, Germany) at 20800 g for 30 min at 20 °C. After phase separation, 0.5 mL of the supernatant was dried under vacuum (concentrator plus, Eppendorf, Germany). Then, the dried compound containing EB was dissolved in 2 mL of methanol, and the total amount of EB was determined by High Performance Liquid Chromatograph (HPLC), Agilent 1260, equipped with UV detector at 245 nm and HPLC column (Zorbax Eclipse XDBC18 (150 × 4.6 mm i.d., 5 µm particle size, stainless steel). The mobile phase consists of acetonitrile (99.8 %): acetic acid (0.1 %) (80:20 v/v). The injection volume was 20 µL with a flow rate of 1.0 mL min^{-1}, the oven temperature maintained at 30 ℃.

The percentage of entrapment efficiency (EE %) of EB in EB+PNC was determined by measuring the concentration of the free unloaded compound in the aqueous phase of the EB+PNC. Centrifugation was carried out using a tube filter containing 0.22 µm pore cellulose acetate membrane (Costar Spin-X, Corning Inc.). A total of 0.5 mL of colloidal EB+PNC suspension was placed in the outer chamber of the filter assembly, and the assembly was then centrifuged at 2700 g for 15 min at 15 °C. The encapsulated compounds were remained in the outer chamber, whereas the aqueous medium containing the free unloaded EB was moved to the sample recovery chamber through the filter membrane. After separation, 0.2 mL of the aqueous medium was dried. The dried product was dissolved in 2 mL of methanol, and subsequent concentration was determined by HPLC as described earlier by (Forim et al. 2013). The % EE was subsequently calculated using the following equation:

$$EE\% = \frac{\text{total quantity of emamectin benzoate} - \text{quantity of free of emamectin benzoate in the aqueous medium)}}{\text{total quantity of emamectin benzoate}} \text{x } 100$$

4.2.3.2 Determination of emamectin benzoate in EB+SNPs and EB+MCM-48

The total amount percentages of absolute recovery (AR %) of EB in the nanoformulations were determined using the following method. First, 1 mL of the EB+SNPs or EB+MCM-48 solution was dissolved in 10 mL of dichloromethane, and the samples were sonicated for 30 min and then the mixture was magnetically stirred at 1000 rpm for 3 h to ensure complete extraction of the EB from samples. After this, 1 mL from the samples were centrifuged (centrifuge 5417 R; Eppendorf, Germany) at 20800 g for 30 min at room temperature. After phase separation, 0.1 mL of the supernatant was dried under vacuum (concentrator plus, Eppendorf, Germany). Then, the dried compound containing EB was dissolved in 1 mL methanol, and the total amount of EB was determined by HPLC as done in EB+PNC.

The percentage of entrapment efficiency (EE %) of EB was determined by measuring the concentration of the free unloaded compound in the aqueous phase of the colloidal solution according to Forim et al. (2013) as previously mentioned above in 4.2.3.1.

4.2.4 Characterization of EB+NFs

The surface morphology was observed using scanning electron microscope (SEM, TM-1000, Hitachi, Japan) and transmission electron microscope (TecnaiTM Spirit TEM, FEI Company, Hillsboro, OR, USA). X-ray diffraction (XRD) measurements were performed using a multipurpose X-ray diffractometer (XRD, X'Pert PRO, PANalytical, Almelo, The Netherlands) with Cu radiation (λ = 1.54 A) at 40 kV and 40 mA. The samples were scanned from 1 to 90 °θ, and the scanning rate was 2 °θ per min with step size of 0.02 °θ. Fourier transform infrared spectrophotometer (FT-IR) (Vector 22, Bruker, Germany) was used to identify the different functional groups presented in the samples. Particle size and zeta potential values were

evaluated by NanoPlus “Particle Size & Zeta Potential Analyzer” (Particulate Systems a division of Micromeritics, 4356 Communications Drive, Norcross GA, 30093, USA).

4.2.5 Stability assay of EB+NFs against UV radiation

The stability of the EB+NFs against ultraviolet (UV) radiation was tested by exposing the samples to a 36 W germicidal lamp (254 nm) at a distance of 20 cm at room temperature. The samples were withdrawn every 12 h in the 72 h for analysis and the changes of the EB content were analyzed by HPLC. The methanol solution of the EB (a.i.) was used as the control sample at the same time (Guo et al. 2015).

4.2.6 Release study

The release experiments were prepared using the method of Guo et al. (2015), 0.1 g from different EB+NFs were weighed and suspended in 100 mL of the methanol–water mixture (30: 70, v/v). This methanol–water mixture was used as a release medium in order to dissolve the EB. 5 ml suspension of the EB+NFs was introduced into a dialysis bags and stirred at a speed of 100 rpm at room temperature, then the released EB from the dialysis bags was monitored up to 72 h. The released solution was collected at different intervals after (2, 4, 6, 12, 24, 36, 48, 60 and 72 h), and centrifuged at speed 10000 rpm for 15 min at room temperature. The concentration in the supernatant was determined using HPLC and the cumulative release rate of the EB from the EB+NFs was calculated to evaluate the sustained release properties. Data of the gradual release curve from EB+NFs release experiments were fitted to the following equation (Korsmeyer et al. 1983; Wang et al. 2015).

$$\frac{M_t}{M_\infty} = kt^n$$

M_t is the amount of emamectin benzoate released at time **t**, M_∞ is the total amount of emamectin benzoate in EB+NFs, **k** is a release constant and **n** is a diffusional exponent.

4.2.7 Insect culture

The initial population of *P. xylostella* was collected from a cruciferous vegetable field in the eastern suburbs of Hangzhou (30°14'N, 120°15'E), Zhejiang Province, China, in September 2014. *P. xylostella* was reared at 25 ± 2 °C with 70 % ± 10 % relative humidity (RH) and a light–dark cycle of 16:8 h. The larvae were reared on cabbage leaves (*Brassica oleracea* var. capitata (L.) (Capparales: Brassicaceae) cv. Jingfeng No. 1), and adults were fed with 10 % sucrose solution (Gu et al. 2015).

4.2.8 Bioassay experiments

The bioassay for the efficacy of EB+NFs against the early third instar larvae of *P. xylostella* was performed in plastic containers (14.2 × 7.2 × 5.2 cm) using leaf dipping method and carried out at 25 ± 2 °C with 70 % ± 10 % RH and a light–dark cycle of 16:8 h. Dilutions of EB+NFs and EB were prepared using ultrapure water. The concentrations were prepared based on the mortality rang falling between 20 % and 80 % (Robertson et al. 1984) and the total volume was 100 ml for each concentration. Leaf discs (2.5 cm in diameter) of cabbage leaves were dipped for 30 s in the test solution with gentle agitation. The leaf discs dipped in ultrapure water were served as control. Thirty min later, the surface of leaf discs was air dried, one dipped leaf disc with 30 early third instar larvae was placed in a perforated plastic container. The larvae were allowed to feed on the treated leaf disc for 24 h, and then

fed them with untreated clean leaf disc every day. Insect mortality was recorded at 24, 48, and 72 h after the larvae were exposed to EB+NFs or EB alone. Three replicates for each concentration were performed, and 30 larvae were utilized for each replicate.

4.2.9 Statistical analysis

The mortality was analyzed via a two-way analysis of variance (ANOVA) with IBM SPSS Statistics 19 software. All percentage data were transformed using arcsine square root before ANOVA to standardize means and normalize variances and were transformed back to percentages for presentation. Mean values were separated through the least significant difference (LSD) test ($P< 0.05$) when significant differences among several mean values were detected by ANOVA. Percentages of mortality were corrected according to Abbott's (1925) formula, and the LC_{50} values were calculated using the statistical method of LdP line program software (http://embakr.tripod.com/ldpline/ldpline.htm), which was devoted to the calculation of probit analysis based on Finney's (1971).

4.3 Results

4.3.1 Encapsulation efficiency of EB+NFs

The HPLC analysis was used to investigation of % AR, % EE, stability, and kinetics release. The standard curve of emamectin benzoate showed a good linear relationship between the concentrations that ranged from 5 to 100 mg L^{-1} with linear equation $y=18.08x-2.2698$ ($R^2=0.9994$) (Figure 5). The AR was 72.51 %, 94.55 % and 74.29 %, and EE of EB+NFs was 92.84 %, 87.45 % and 71.19 % from EB+PNC, EB+SNPs and EB+ MCM-48, respectively (Table 4).

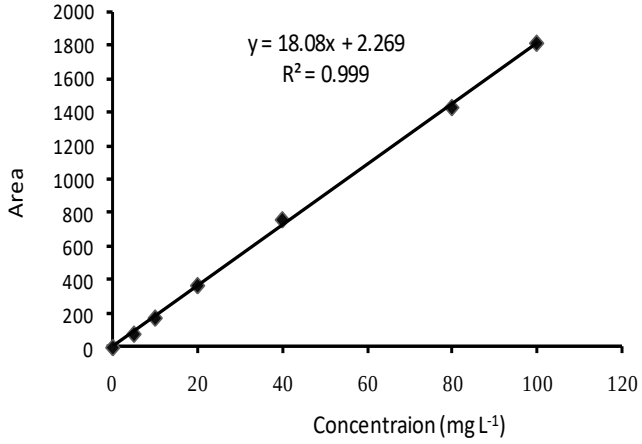

Figure 5: Standard curve of emamectin benzoate.

4.3.2 Characterization of the EB+NFs

4.3.2.1 Analyses of morphology and sizes of EB+NFs

Regarding to EB+PNC, according to TEM micrographs, the EB has been successfully encapsulated in the nanocapsules of ethyl cellulose (Figure 6) and the nanocapsules were of spherical in shape with an average size of 219.93 ± 3.89 nm (Table 4). SEM micrographs also revealed that the EB+PNC had a homogeneous distribution of particles (Figure 6). Regarding to EB+SNPs, TEM image showed that the SNPs had nanometric sizes (Figure 7). The SNPs had spherical shape with a small percentage of irregular surfaces, TEM image showed that the EB adsorbed by SNPs and created thin film around the SNPs (Figure 8), and the average size was around 142.77 ± 3.43 nm (Table 4). The TEM image of the MCM-48 is presented in Figure 9. The TEM micrographs showed that core–shell-structured silica nanoparticles, with an average size of 119.73 ± 20.28 nm (Table 4) and a shell thickness of 10 ~ 15 nm. The EB was incorporated in pores of MSNs to obtain controlled release formulation. The pore size of MCM-48 wall is larger than the diameter of EB, allowing EB molecules to be entrapped into the MCM-48 (Figure 10).

Table 4. Characterization of EB+NFs.

Formulation	Absolute recovery AR (%)	Entrapment efficiency EE (%)	Particle size (nm)) ± SD	Zeta potential (mV) ± SD
EB+PNC	72.51	92.84	219.93 ± 3.89	-26.43 ± 2.90
EB+SNPs	94.55	87.45	142.77±3.43	-41.00±1.31
EB+MCM-48	74.29	71.19	119.73 ± 20.28	-36.50 ± 0.56

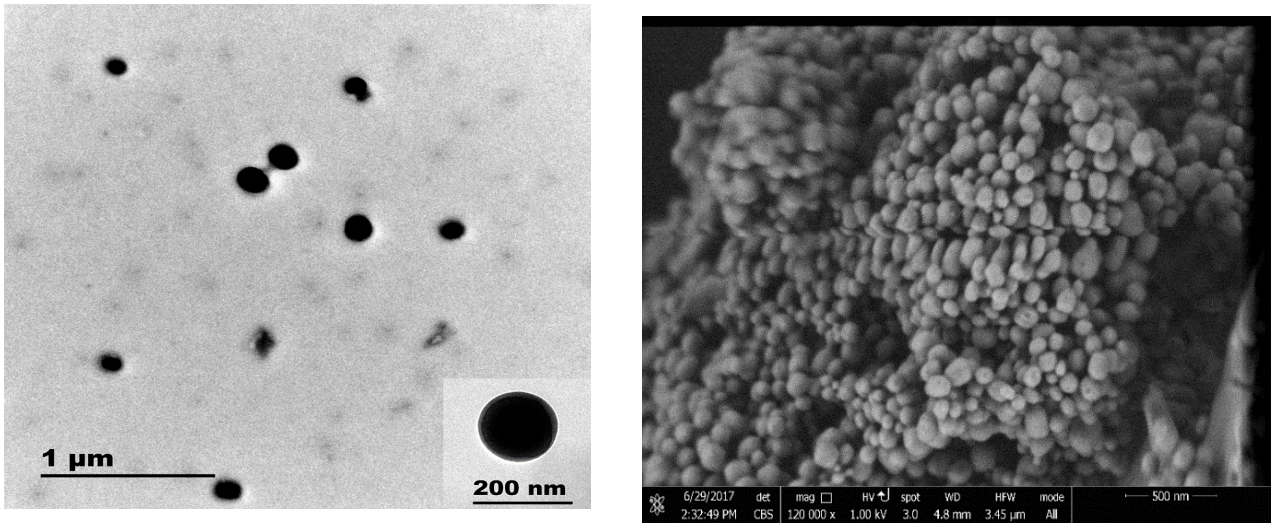

Figure 6: TEM and SEM image of EB+PNC.

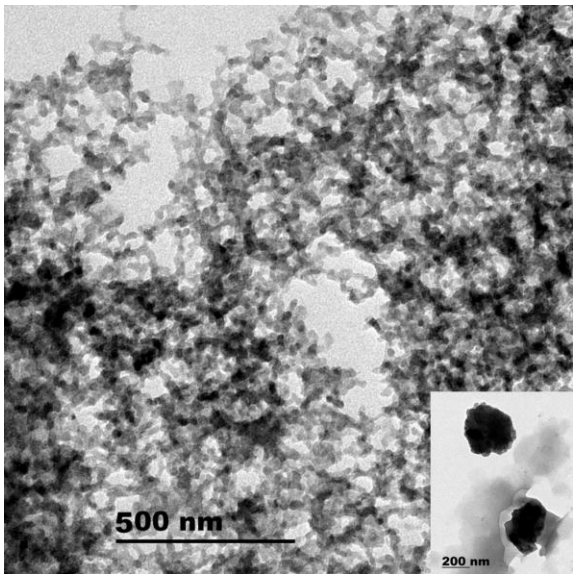

Figure 7: TEM image of SNPs.

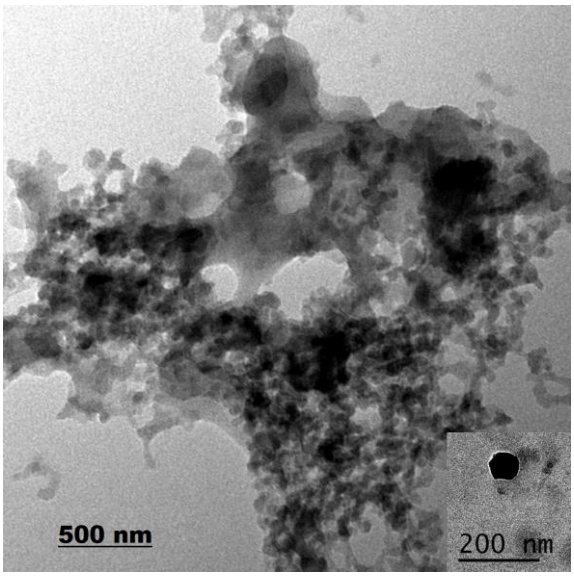

Figure 8: TEM image of EB+SNPs insecticide.

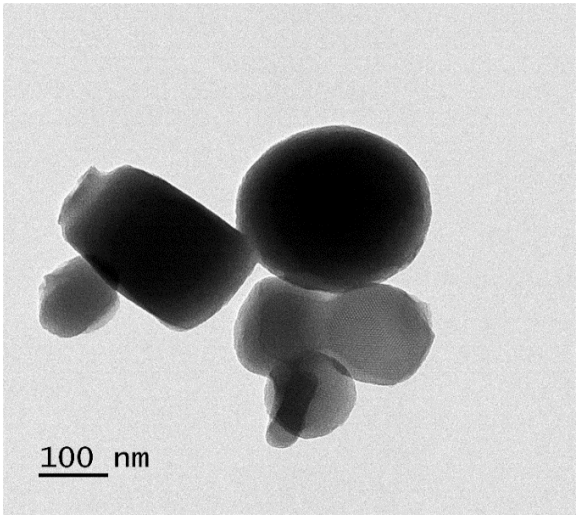

Figure 9: TEM image of MCM-48.

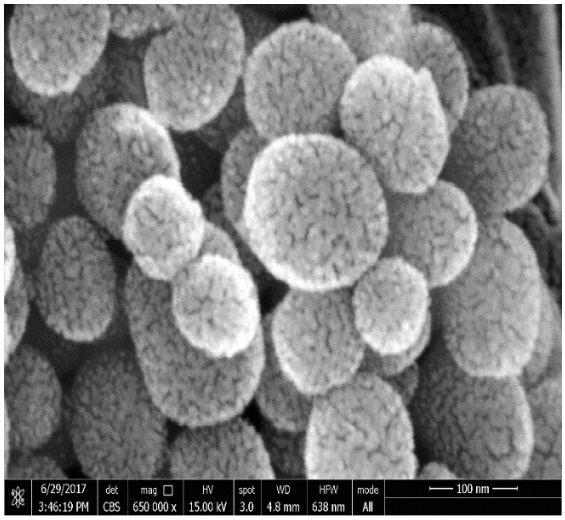

Figure 10: SEM image of EB+MCM-48.

4.3.2.2 XRD analysis

The crystal structure of SNPs and MCM-48 (Figure 11) showed the XRD pattern of SNPs and MCM-48, the SNPs peak was observed at 21.218 °θ using Bragg's law, that is, λ = 2dSin θ and the MCM-48 peak was observed at 2.6°θ. These results showed a broad peak for an amorphous nanosilica core region.

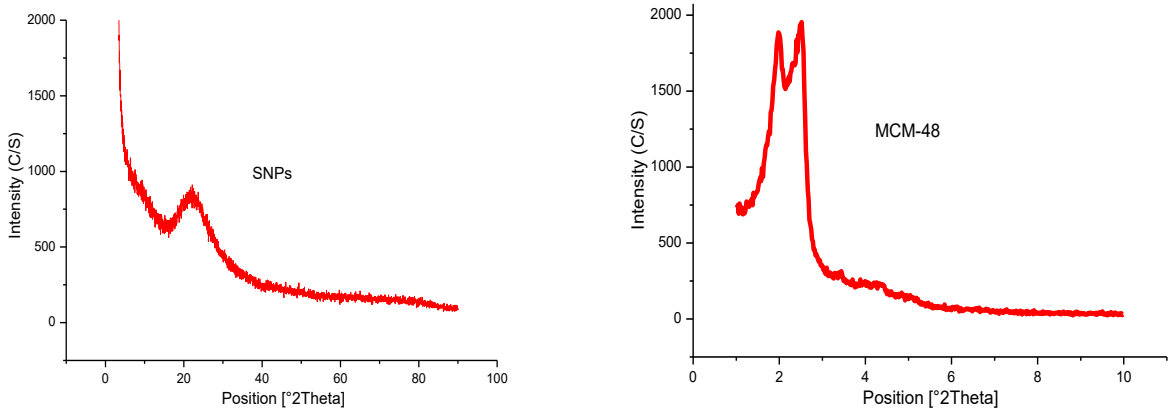

Figure 11: XRD pattern of SNPs and MCM-48 nanosilica show a single broad peak for an amorphous nanosilica core region.

4.3.2.3 FT-IR analysis

The infrared spectra of EB blank, SNPs, MCM-48, EB+SNPs and EB+MCM-48 samples are shown in (Figure 12 and Figure 13). Regarding to SNPs, the absorption band at 3419 cm $^{-1}$ (Figure 12 A) showed that only a small amount of water is present in the samples. The very broad strong peak at 1097 cm^{-1} can be ascribed to composite of Si-O stretching of nanosilica (Figure 12 B). For the free EB, the bands at 2967 and 2982 cm $^{-1}$ are attributed to (C-H stretching vibrations of an aromatic ring corresponding to the benzoate fraction or conjugated olefins), 1716 cm^{-1} bending vibration of (C=O stretching vibrations of an arylic ester), 1634, 1599 and 1557 cm^{-1} are ascribed to (C=C stretching vibrations of an aromatic ring or conjugated olefins), 1455 and 1379 cm^{-1} are identified as skeleton vibration of (C-H deformation in CH3

groups), 1160, 1118 and 1058 cm^{-1} are attributed to (C-O stretching vibrations, O-H and C-O-C flexion), 991 cm^{-1} bending vibration of (C-H flexion of trans C=C bonding) and 947 – 568 cm^{-1} (C-H flexion outside the plane in an aromatic ring or C=C cis bond) (Figure 13 C). For the EB+SNPs, the spectrum retained most of the major peaks of SNPs and EB, and no noticeable new peaks were observed in EB+SNPs (Figure 12 A). The FT IR of free EB blank MCM-48 and EB+MCM-48 showed in Figure 13, the obtained results for blank MCM-48 is similar with SNPs spectrum that mentioned above, The very broad strong peak at 1090 cm^{1} can be ascribed to composite of Si-O stretching of nanosilica (Figure 13 B).The FT IR of EB was mentioned above and no noticeable new peaks were observed EB+MCM-48 (Figure 13 A).

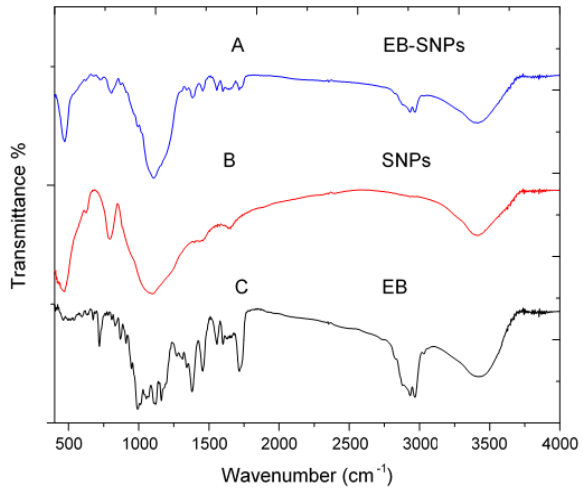

Figure 12: FTIR spectrum of SNPs and EB+SNPs.

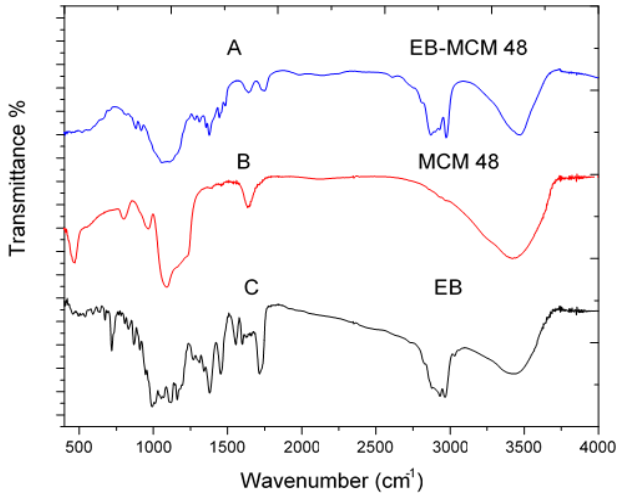

Figure 13: FTIR spectrum of MCM-48 and EB+MCM-48.

4.3.2.4 Zeta potential

The zeta potential degree describes the electrostatic repulsion degree between adjacent, similarly charged particles in dispersion. The colloidal solution with ZP of > +30 mV or < −30 mV is considered to be very stable. The zeta potential values for all EB+NFs were fallen in the range of −26 to −41 mV (Table 4).

4.3.3 The effects of UV radiation on the stability of the EB+NFs

The photo-stability of EB+NFs and the EB alone are shown in the Figure 14. The degradation rates were 15.35, 34.94, 52.58 and 59.50 % for EB+PNC, EB+MCM-48, EB and EB+SNPs after exposure of 72 h, respectively. These results showed that the EB+PNC was more stable than all other samples, and no significant difference was detected between EB and EB+SNPs. The UV radiation results showed that the EB could be protected through the EB+PNC and EB+MCM-48, nanosilica wall can significantly prevent the photolysis of EB and increase the EB stability. While the EB+SNPs did not protect EB from photolysis because EB was only adsorbed on the surface of SNPs.

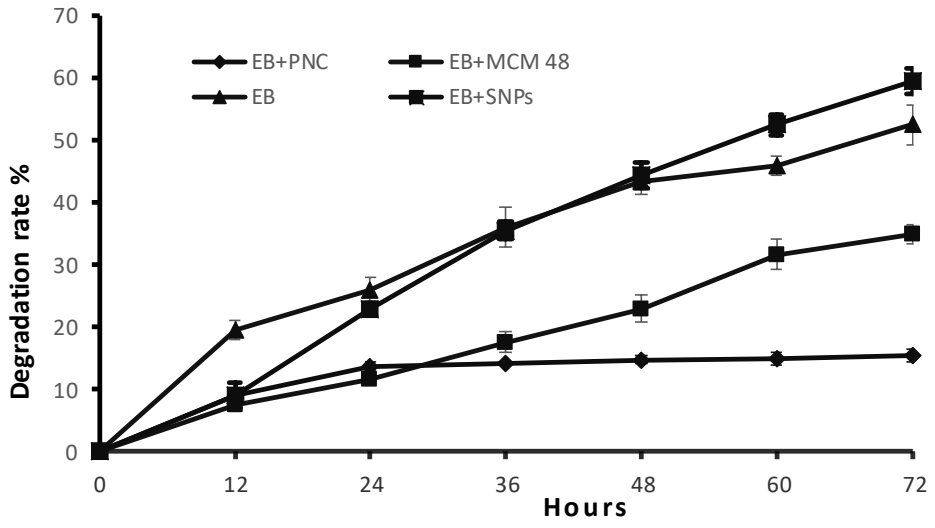

Figure 14: Stability of EB+NFs and active ingredient against UV radiation. The UV radiation results showed that EB could be protected through EB+PNC and EB+MCM-48, but no significant difference between EB and EB+SNPs.

4.3.4 The release behaviors of EB+NFs

The release profiles of EB+NFs showed in the Figure 15. The releases of EB in all samples were relatively fast at initial stage and then progressively slow with

increasing time. The cumulative release rates were 10.11, 25.84, 45.77 and 50.47 % after 72 h for EB+MCM-48, EB+PNC, EB and EB+SNPs, respectively.

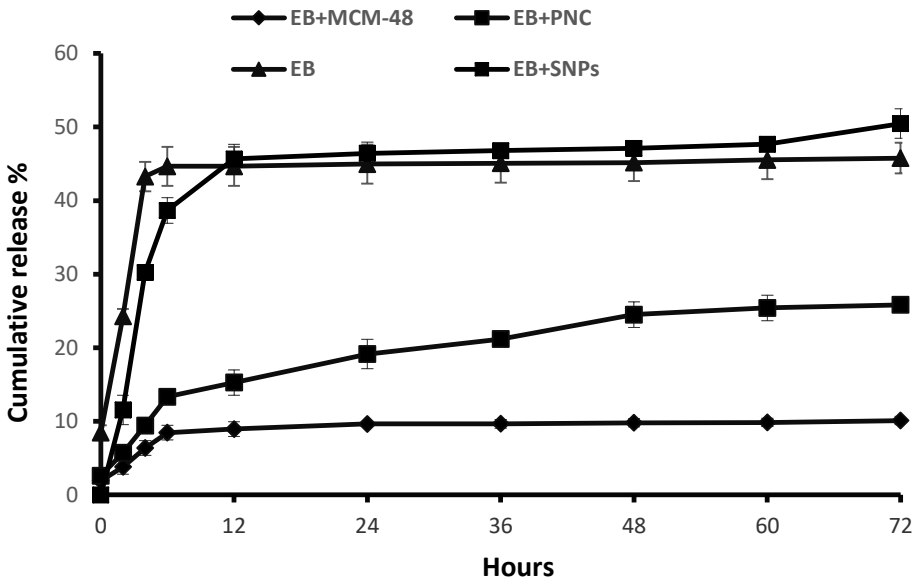

Figure 15: The release behaviors of the EB and EB+NFs.

4.3.5 Bioassay results

All main effects and their associated interactions were significant at P <0.0001 level (Table 5).

In all treatments, mortality percentage increased with the increase in concentrations and with the passage of time (Table 6). The efficacy of EB+NFs was compared on the LC_{50} values. Results showed that the EB+SNPs is more effective than other treatments because it showed 100 % mortality after 48 h but other treatments EB+MCM-48, EB and EB+PNC showed 86.67 %, 88.86 % and 84.44 % mortality after 72 h respectively (Table 6) The lowest LC_{50} was recorded when larvae were exposed to EB+SNPs, followed by EB+MCM-48 which are much lower than EB+PNC and EB. However, no significant difference was observed between the EB+PNC and the EB. The LC_{50} value of EB+SNPs was 0.18 mg L^{-1} after 48 h, while

the LC_{50} values for other treatments were 1.8, 4.22 and 4.52 mg L^{-1} of EB+MCM-48, EB and EB+PNC after 72 h, respectively (Tables 7).

Table 5: Analysis of variance of the main parameters and their interactions.

Source	EB		EB+PNC		EB+SNPs		EB+MCM-48	
	F value	P value	F value	P value	F value	P value	F value	P value
Time	259.62	0.0001	428.39	0.0001	288.55	0.0001	77.57	0.0001
Concentration	392.98	0.0001	580.83	0.0001	1776.89	0.0001	221.82	0.0001
Time* Conc.	21.12	0.0001	32.36	0.0001	20.07	0.0001	4.80	0.0001

Table 6: Mortality (mean ± SD) of the third instar larvae of *P. xylostella* after EB+NFs exposure via leaf dipping.

Formulation	Concentration (mg L^{-1})	hours after treatment		
		24 h	48 h	72 h
EB+SNPs	0.1	14.77 ± 1.92 e	27.27± 1.92 e	34.09 ± 1.92 c
	0.2	31.81 ±3.33 d	52.27±3.33 d	65.91 ±3.33 b
	0.4	62.43 ± 3.33 c	79.54 ±3.33 c	100.00 ± 0.00 a
	0.8	76.14 ± 3.33 b	86.36 ± 3.33 b	100.00 ±0.00 a
	1.6	86.36 ± 3.33 a	100.00 ± 0.00 a	100.00 ±0.00 a
	control	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 d
EB+MCM-48	0.5	10.00 ± 1.92 d	13.33 ± 3.33 d	22.22 ± 5.09 e
	1	14.44 ± 1.92 cd	21.11 ± 3.85 cd	38.89 ± 5.09 d
	2	21.11 ± 5.09 c	26.67 ± 6.67 c	47.78 ± 5.09 c
	4	37.78 ± 5.09 b	45.56± 3.85 b	64.44 ± 6.94 b
	8	53.33 ± 8.82 a	72.22 ± 10.18 a	86.67 ± 3.33 a
	control	0.00±0.00 e	0.00±0.00 e	0.00±0.00 f
EB+PNC	1	4.44±0.00 cd	11.11±3.33 d	17.77±3.33 d
	2	5.56±1.92 c	14.44±1.92 d	26.67±1.92 d
	4	8.89±1.92 c	25.55±3.33 c	41.11±3.33 c
	8	17.78±1.92 b	43.33±1.92 b	60 ±3.85 b
	16	37.78±3.85 a	73.33±3.85 a	88.86 ±3.83 a
	control	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e
EB	1	5.56±1.92 de	11.11±1.92 d	16.67±3.33 e
	2	8.89±3.85 cd	14.44±1.92 d	24.44±1.92 d
	4	13.33±3.33 c	23.33±3.85 c	51.11±3.85 c
	8	24.44±1.92 b	41.11±1.92 b	67.78±5.09 b
	16	42.22±3.85 a	62.22±5.09 a	84.44±5.09 a
	control	0.00±0.00 e	0.00±0.00 e	0.00±0.00 f

Table 7: $LC_{50–90}$ values of EB+NFs against the third instar larvae of *P. xylostella.*

Formulation	Time (h)	LC_{50} (mg L^{-1}) (LCL–UCL)	LC_{90} (mg L^{-1}) (LCL–UCL)	Slope ± SE	x^2
EB+SNPs	24	0.32 (0.27 – 0.37)	1.67 (1.26- 2.47)	1.78 ± 0.16	2.66
	48	0.18 (0.15 – 0.21)	0.75 (0.60 – 0.99)	2.07 ± 0.19	3.47
EB+MCM-48	24	7.44 (5.37 – 12.24)	89.03 (40.26 – 347.38)	1.18 ± 0.16	1.23
	48	4.03 (3.23 – 5.34)	33.81 (19.96 – 76.32)	1.38 ± 0.16	5.05
	72	1.80 (1.46 – 2.21)	14.27 (9.61 – 25.64)	1.42 ± 0.15	3.32
EB+PNC	24	34.79 (21.51- 82.29)	359.51 (131.92- 2392.08)	1.26 ± 0.22	3.23
	48	8.49 (7.00 – 10.74)	55.23 (35.88 – 103.62)	1.57 ± 0.16	3.82
	72	4.52 (3.83 -5.37)	26.30 (19.05 – 41.02)	1.67 ± 0.15	7.45
EB	24	24.83 (17.41- 56.21)	311.32 (119.88 – 1783.47)	1.20 ± 0.18	1.32
	48	11.06 (8.56 – 15.75)	99.64 (53.95 – 265.99)	1.34 ± 0.16	2.70
	72	4.22 (3.55 – 5.04)	24.14 (17.46 – 38.08)	1.69 ± 0.16	1.70

LCL: lower confidence limit and ULC: upper confidence limit.

4.4 Discussion.

4.4.1 Encapsulation efficiency (EE)

The ethyl cellulose, SNPs and MCM-48 were used to improve the EB formulations to avoid the disadvantage and the side effects of pesticides. According to the obtained EE % results, the NFs preparation method is suitable for preparation of EB+NFs.

Ethyl cellulose that are used in preparation of EB+PNC showed a higher encapsulation rate than other nanocapsule formulations, this may be due to its physical and chemical properties and its high ability to encapsulate the active ingredient in the nanocapsules (Fernández-Pérez et al. 2011; Rao and Murthy 2002).

The EE % of MCM-48 for EB reached to 71.19 %. The efficacy of MCM-48 for EB adsorption is due to its large surface area, porous structure and small particle size. The pore diameter also plays an important role in the loading process. The MCM-48 channels porous diameter is larger than the sectional diameter of EB molecules, allowing EB molecules to be entrapped into the porous structure of MCM-48 nanosilica. Furthermore, the type of MCM-48 nanosilica has a 3D cubic mesoporous structure with open networks and high surface area (Popat et al. 2012). This structure increases its adsorption capacity of the EB. Our results support several earlier studies. The amount of avermectin encapsulated in the MSNs reached 58.3 % w/w by a simple immersion loading method, thus most of the adsorption of avermectin on the mesoporous nanosilica might be physical (Wen et al. 2005). The MSNs with a shell thickness of ∼15 nm and a pore diameter of 4–5 nm have an encapsulation capacity of 625 g kg^{-1} for avermectin using a supercritical fluid loading method (Sayyed and Wright 2006). Similarly, MCM-48 nanospheres can be utilized as an effective delivery carrier owing to their high surface area and unique 3D open pore structure (Popat et al. 2012). In addition, the MSNs showed excellent pesticide loading capacity and delivery performance in controlled release, anti-photolysis, and water dispersity of abamectin (Wang et al. 2014).

Pesticide loading can be achieved by different methods on the nanoparticles carriers such as extrusion, spray dry and freeze dry (Perlatti et al. 2013). A successful colloidal delivery system should have a high loading capacity. In this study, the EE of EB+SNPs reached to 87.45 %, the high loading capacity maybe due to the competition between its solubility in the water and its adsorption on to the SNPs surface. Also, because the EB is poorly soluble in aqueous media, and its ability to be adsorbed on the SNPs is higher than its solubility in the water. In addition, silica gel is a widely employed compound in the column chromatography as a stationary phase

and as adsorbents in the environmental studies because it has high adsorption potential of organic and inorganic compounds (Kushwaha et al. 2017).

4.4.2 Characterization of the EB+NFs

The images of SEM and TEM showed that the EB has been successfully encapsulated in the nanocapsules of ethyl cellulose polymers and successfully loaded with MCM-48 and SNPs. The EB+NFs showed a relative homogeneous in size and shape, the mean particle size was 219.93, 142.77 and 119.73 nm for EB+PNC, EB+SNPs and EB+MCM-48 respectively. The well-known ethyl cellulose is one of the most constructive polymer, and is used widely to synthesize the microcapsules and nanocapsules for drugs and pesticides because of its advantages as formulator, such as perfect film formability, higher physical–chemical stability, and minimum toxicity (Fernández-Pérez et al., 2011; Latheef et al., 1993; Lokhande et al., 2013; Rao and Murthy, 2002). Regarding MCM- 48, the MCM-48 has an average diameter of 119.73 ± 20.28 nm with a spherical in morphology and a highly organized porous structure, and it has similar characteristic reported by Popat et al. (2012). The freeze-drying technique is used to enhance the stability of colloidal nanoparticles which also include nanocarriers for CRFs of pesticides. It is widely used for drying the unstable or heat-sensitive compounds at low temperatures without damaging their chemical structure. In this study, the SNPs colloidal solution mixed with EB (a.i.), then the aqueous phase was frozen and subjected to a low-pressure system. When the pressure is reduced drastically, the water sublimates (goes from solid to vapor state) and the EB a.i. leaves on SNPs.

The XRD diffraction patterns for the both crystalline SNPs and MCM-48 showed a broad peak at 21.218 °θ, and 2.6 °θ respectively, which indicates its amorphous silicon dioxide. The XRD pattern of SNPs was in good agreement with the earlier

reported findings (Debnath et al. 2012b; Vani and Brindhaa 2013). Both studies reported that the peak was approximately at 20 °θ. With regards to MCM-48 nanosilica, the obtained results established that the synthesized compound is MCM-48 nanosilica. This result was in accordance with Choi et al. (2014), they observed a sharp first Bragg peak indexed as (211) at 2 °θ = 2.51°θ and the second peak (220) at 2.89 °θ for the cubic *Ia*3*d* mesostructure (MCM-48). It is also compatible with previous studies which discussed the chemical and physical characterization for the MCM-48 nanosilica (Schumacher et al. 2000; Trikalitis et al. 2004; Izquierdo-Barba et al. 2009).

Zeta potential is an excellent technique for describing the properties of the nanoparticle surface and predicting the long-term existence of the nanoparticle. In the low zeta potential, attractive forces may overcome this repulsion and the dispersion may split and flocculate. So, the colloidal system with high zeta potential are electrically stabilized while the colloidal system with low zeta potentials are electrically unstable (áO'Brien, 1990; Hanaor et al., 2012). Especially, the NPs with zeta potential of > +30 mV or < − 30 mV is considered to be very stable in the dispersion medium (Forim et al., 2013; Bhattacharjee, 2016). In our study, the zeta potential values for all EB+NFs were more than −26 mV, suggesting that the NPs formulations were stable in the dispersion medium.

The FT-IR analysis was employed to examine possible interactions between the EB and the nanosilica carriers. The FT-IR results confirmed that the EB+NFs spectrums retained most of the major peaks of pure carriers (SNPs and MCM-48) and EB, and did not show noticeable new peaks, indicating that the adsorption of EB in the NPs carriers is probably physical adsorption. Therefore, the properties of EB have not been changed after their loading on the NPs carriers. These results were convenient with the absorption behavior of avermectin–PHSN as reported by Wen et al. (2005).

4.4.3 Stability of EB+NFs to the photodegradation

Protecting the a.i. of a formulation under field conditions is necessary when the local environment adversely affects the stability of the pesticides. So, the encapsulation is necessary to overcome the stability problems of a.i. and also to improve the solubility of this pesticide in water (Perlatti et al. 2013).

Regarding EB+PNC, the ethyl cellulose enhanced the photostability of EB+PNC, the stability against UV radiation maybe due to the physical and chemical characteristics of ethyl cellulose. The ethyl cellulose has been widely used for microencapsulation due to its versatile properties such as melting point range 240 - 255 °C, specific density range 1.07 - 1.18 with 135 - 155 °C heat distortion point and 330 - 360 °C fire point, stability against light, heat, wetness and chemicals, and ability to absorb pressure (Murtaza 2012). Because, the polymeric chain forms a stronger film isolating the a.i. from the external environment, it can protect the a.i. from the degradation by UV. The previous studies confirmed that the polymeric nanocapsules could improve the persistence of a.i. against UV radiation such as natural products (Forim et al. 2013). On the other hand, the acetamiprid microcapsules prepared with tapioca starch, urea, and sodium borate were more stable than the acetamiprid commercial emulsifiable concentrate (EC) against UV radiation and high temperature storage (Cao et al. 2005). Also, the emamectin-benzoate slow release microspheres showed excellent anti-photolysis performance, stability, controlled release properties, and good leaf distribution (Wang et al. 2017). Consequently, the polymeric nanocapsules is able to protect the a.i. from the rapid degradation or may increase the efficiency of pest control for a long duration. Furthermore, it can be able to lower the dosage of pesticides and exposure to human.

The EB+MCM-48 reduced significantly the degradation rate of EB. This is probably due to the EB was entrapped into the pores of MCM-48. These results were

consistent with the previous study by Guo et al. (2015), they found that the EB was sensitive to UV radiation and the samples were degraded completely within 48 h, while the decomposition rate of the EB wrapped in microcapsules was less than 25 % after 72 h of UV exposure. On the other hand, the MSNs highly improved the photostability of avermectin by entrapping it into the hollow core of the nanoparticle carriers (Wen et al. 2005; Li et al. 2007). The MSNs not only can protect the active ingredient from UV radiation but also can enhance the chemical solubility and its dispersity in the water (Wang et al. 2014). Improvement of the insecticides stability can reduce the concentration of insecticides in commercial spray applications, without lowering the efficiency. These kinds of the formulations (EB+PNC and EB+MCM-48) are convenient for application in the early stage of plant life, which requires stable pesticides under various environmental conditions to protect the plant for long period. Consequently, they may reduce economic cost by decreasing the number of applications. Moreover, insecticide nanodelivery systems were proposed to increase the spatial distribution on leaf surfaces of crops due to the nanosize and thereby enhance the effectiveness of pesticide applications. Besides, pesticide nanodelivery systems also have better penetration ability through the cuticle, and allow slow and controlled release of active ingredients on the target (Wang et al. 2014).

The EB and EB+SNPs showed no significant difference in the stability against UV radiation. The SNPs do not have the ability to protect the a.i., this may be due to that the active ingredient in EB+SNPs were adsorbed on the surface of the SNPs and exposed directly to UV radiation.

4.4.4 The release behaviors of EB+NFs

CRFs play a critical role to reduce the environmental problems associated with the application of pesticides. The colloidal delivery system has great potential to improve the pesticide CRFs and remarkably reduce effective dosage by maintaining an effective concentration in the target for longer periods of time (Wang et al., 2014). In this study, the EB+MCM-48 showed two stages in release behavior. The release in the first few hours was so fast, then, the release rate became much slower in the later hours. In the first stage, it may be due to the dissolvent of the EB adsorbed on the external surface of MCM-48, while in the second stage, it may be due to the hindrance of the porous structure of MCM-48. These results showed good agreement with the release behaviors of avermectin porous hollow silica nanoparticles (avermectin-PHSNs), abamectin-PHSNs and imidacloprid-mesoporous silica nanoparticles formulations (Wen et al. 2005; Popat et al. 2012; Wang et al. 2014).

The EB+PNC release behavior was similar to that of EB+MCM-48 release. The slow release profile may be due to the stronger mechanical property of the nanocapsules that prevent the excessive release of EB from the EB+PNC. Moreover, the denser ethyl cellulose polymer chains and its physicochemical properties led to the reduction in release (Rao and Murthy, 2002; Fernández-Pérez et al., 2011). The release profiles of EB both in EB+SNPs and in the control (EB alone) showed no significant difference. Both showed a faster release at first 12 h, this may be due to the fast dissolution of EB on the surface of SNPs.

4.4.5 Bioactivity of EB+NFs

The EB+SNPs was most effective in all four treatments. The lowest LC_{50} was EB+SNPs, followed by EB+MCM-48 which are much lower than EB+PNC and EB. However, no significant difference was observed between the EB+PNC and the EB.

The improved efficacy of EB+SNPs and EB+MCM-48 may be due to the smaller particle size, higher surface area and their high mobility ratio, which eventually lead to increasing penetration of NPs formulations in the larval body than the active ingredient alone. The surface-functionalized silica nanoparticles can deliver DNA and drugs into animal cells and tissues (Torney et al., 2007), because nanoparticles drug carriers have the potential to cross physiological barriers and access different tissues (Rosenholm et al., 2010). The Functional Nano- Dispensers (FNDs) of imidacloprid was an approximate 200 fold reduction in the amount of imidacloprid required to cause similar mortality of *Diaphorina citri* as compared with the commercial formulation. Such a reduction in insecticide concentration, while maintaining similar efficacy, shows promise to significantly reduce negative environmental impact of this important pest management tool (Meyer et al., 2015). The insecticidal activity of pyridalyl nanosuspension was more effective than the commercial formulation and was 2.26 and 6.25 times more effective against *H. armigera* as stomach poison than the technical product and commercial formulation respectively (Saini et al., 2014). They thought that the increased toxicity of nano sized formulation on larvae is probably due to increasing penetration of pyridalyl in the larval body. Regarding to the effectiveness of EB+PNC and EB, a little difference was noticed between their effectiveness. At the equal concentration, EB showed more effective than that of the EB+PNC in the first day, whereas EB+PNC was more effective than EB alone during the second day. These results may be due to the efficacy of EB+PNC is dependent on the controlled release of the a.i. from the nanocapsules. These results are consistence with the findings of Guo et al. (2015), who reported that when treated *M. persicae* at the same concentration, EB 1 % EC is more effective than that of the microcapsules at first day after treatment. Similar results were also reported by Zhang et al. (2016), where the effectiveness of phoxim

microcapsules increased with the passage of times. The similar results could be due to that the insecticides were loaded on the similar carrier with similar physicochemical properties.

In conclusion, according to our study, we can suggest that the colloidal delivery systems such as SNPs, MCM-48 and PNC could act as a controlled release carrier and can maintain chemical stability of EB. They may overcome environmental sensitivity and poor water solubility and increase the efficacy of insecticides. These advantages could eventually lead to minimize the dosage of pesticides needed, reducing the number of applications required in comparison to conventional formulations and decreasing pesticides release in the environment. However, there are necessaries to study the safety issues regarding pesticide nanoformulations on the beneficial insects and human health.

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List of Abbreviation

3D	Three dimensional	**nm**	Nanometer
a. i	Active ingredient	**NMs**	Nanomaterials
abamectin-PHSNs	Abamectin with porous silica	**NPs**	Nanoparticles
AgNP	Silver nanoparticles	**NSF**	Nanospheres formulation
ANP	Aluminum oxide nanoparticles	**NSA**	Nanostructured alumina
CRFs	Controlled release formulations	**PEG**	Polyethylene glycol
DBM	Diamondback moth	**PHSNs**	Porous hollow silica nanoparticles
DE	Diatomaceous earth	**PN**	Polymeric Nanocapsules
EB	Emamectin Benzoate	**PNS**	Polymeric Nanospheres
EB+MCM-48	Emamectin Benzoate MCM-48	**PVA**	Polyvinyl alcohol
EB+NFs	Emamectin Benzoate Nanoformulations	**SD**	Standard Deviation
EB+PNC	Emamectin Benzoate Polymeric	**SE**	Standard Error
EB+SNPs	Emamectin Benzoate SNPs	**SNPs**	Silica nanoparticles
EC	Emulsifiable Concentrate	**TEM**	Transmission Electron Microscope
FE-SEM	Field Emission Scanning Electron	**TNP**	Titanium dioxide nanoparticles
FNDs	Functional Nano-Dispensers	**ULC**	Upper confidence limit.
FTIR	Fourier-Transform Infrared Spectroscopy	**USDA**	United States Department of Agriculture
LC	Lethal Concentration	**UV**	Ultraviolet
LCL	Lower Confidence Limit	**XRD**	X-ray diffractometer
LSD	Least Significant Difference	**ZNP**	Zinc oxide nanoparticles
MCM-48	Mesoporous nanosilica type MCM-48	**ZP**	Zeta potential
MSNs	Mesoporous silica nanoparticles		