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*A Review*

# Biofabricated nanoparticles: Their delivery and utility in *Plutella xylostella* management

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The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most serious cosmopolitan pests of cruciferous plants. It causes severe damage to crop quality and production, with estimated global losses of 20-40% per year. Consumer preference regarding pest-free and uncontaminated crop, unavailability of highly competitive natural enemies as well as migratory nature of DBM has obligated for the mandatory use of insecticides. The over reliance and indiscriminate use of chemical insecticides to control DBM has lead to development of resistance against almost all the modern insecticides. In addition, it has also developed resistance against most of the *Bt* strain. To overcome the problem of resistance, there is an urgent need for new and highly efficient method to control DBM which must be eco-friendly, cost effective and has minimum impact on the environment. One of such alternatives is nanotechnology which fulfil all the criteria to become an ideal insecticide or delivery system for insect pest management especially DBM. Nanotechnology offers great improvement in the field of pesticides as it is less toxic, shelf-life enhancement and higher water solubility. Loading of Indoxacarb on nanoparticles are reported to be highly effective they suppressed the activity of detoxification enzymes such as GST, CarE, and P450. Green synthesis of AgNPs, Single walled carbon nano tube (SWCNT) also exhibits larvicidal and pupicidal effect. These are also reported to adversely affect food consumption, growth, pupation and fecundity of *P. xylostella.*These nanoparticles have been found to be more stable, also the controlled release of active ingredient for a long time and provides target specific control of *P. xylostella* for solving adverse situations ofthe crop fields like less food security, lesser food productivity and environmental imbalance.

**Keywords**: Biofabrication, Cruciferous plant, Nanoparticles, Nanopesticides, *Plutella xylostella*

Worldwide *Plutella xylostella* caused an enormous crop loss of 90% yields and annual damage upto $4-5 billion per year. Management of the pest involves the cost of US $1.0 billion per year[1].The conventional procedure like IPM used in crop fields are inadequate and utilization of huge amounts of chemical pesticides have negative effects on human beings and animals apart from the reduce in fertility of soil[2]. Therefore, without harming the nature, nanotechnology has provided green and effectual liberty for crop protection in agriculture. Nanobiotechnology has been provided options to overcome pesticides, like decreasing toxicity, shelf-life enhancement and by making less water-soluble pesticides to water-soluble pesticides, even more of the positive environmental impacts. Nanotechnology has great involvement in the development of opportunities to deal with complex technical and economic issues of insect pest management in agriculture field. Nanotechnology has developed in the last decade and has been able to create multiple creative materials with a huge range of forthcoming applications[3]. Nanoscale structures like nanomaterials and agrochemical entrapment of pesticides, fertilizers, herbicides, regulatory components of plant growth via involvement of surfactants, surface ionic attachments, dendrimers, polymers and other related components can be utilized in plant protection inputs using rapid delivery; it will ensure the rate of uptake which would be lower for active ingredients and directly reduces the quantity of agrochemical application by reduction of the input[4] (Fig. 1). In terms of environment, the degradation prominence of nanotechnology delivered systems in crop fields is due to solubility and stability improvements[5-8].Certain corporate companies have already been established in the marketing field to produce nano-scale emulsions in the form of microencapsulated pesticides because of the environmental trigger, loading of nanoparticles

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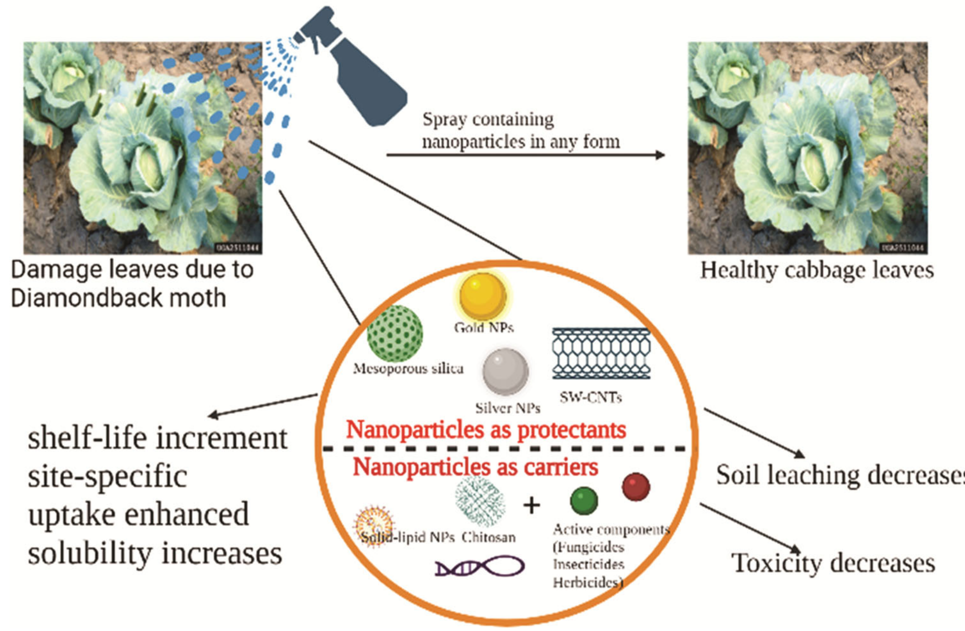


Fig. 1 — Use of nanoparticles for the management of *P. xylostella*

entrapment with pesticides (with slower release in the field), low dose of nanocides products found to be effective in agricultural field protection and their lofty reactivity at nanoscale compared to their mass counterparts[9].

A lepidoptera insect pest of cruciferous plants, *P. xylostella* (Lepidoptera: Plutellidae) is a cosmopolitan and notorious pest worldwide. In the fields, this pest causes 90% of the total yield loss. DBM larvae feed voraciously on leaves of cruciferous plants and causes major damage upto the level of foliar tissue of the leaf veins. The damage of larval stages of diamond back moth (DBM) could be seen in the plants without seedlings and head disruption in broccoli, cabbage, and cauliflower. The florets infested with larvae leads to complete deterioration of product upto the level of plant tissue removal. Diamondback moth was long considered a relatively insignificant minor pest. Its impact was overshadowed by such serious defoliators as imported cabbageworm. Many control measures are accepted for the management of this pest; whereas the most powerful technique to control the damage has involved the heavy dose of chemical insecticides[1]. Overuse of synthetic chemicals proved to cause adverse effects such as insecticidal resistance, environmental imbalance due to their long persistence in the surroundings. According to the present scenario, the urgency evolved to develop an alternative method for control of this insect pest simultaneously this method should be environmental friendly, more effective and provide safer prospective for the economy. The feasible alternative and evolving methods included formation of biofabricated nanoparticles with the help of plant or the plant parts either in the form of extracts or any active compounds for the protection of the field crops[10]. Extra cts of several plants such as bitter gourd, clove, neem, bakain, eucalyptus, datura, ginger and garlic were used for biofabrication of silver nanoparticles and found very effective against *P. xylostella* larvae. The present review has focused on two main aspects of nanoparticles where NPs were synthesized and utilized for management of insect pest *P. xylostella*, either in the form of nanoparticles itself or as carriers for the targeted delivery system and protectants entrapping fungicides, insecticides and herbicides.

## Insecticidal activity of nanoparticles

### Silicon dioxide nanoparticles ($SiO_2$NPs)

The nanosilica exhibited enormous entomotoxic effects on the *P. Xylostella* larvae. Biologically derived nano-silica from wild sugarcane, *Erianthus arundinaceus* showed insecticidal activity against larvae of diamond back moth, *P. xylostella* with $LC_{50}$-30.03 µg/mL[11]. The nanosilica prepared through sol-gel technique and mortality of insect larvae was demonstrated by spray methods, leaf dipping, larva dipping and dust spray methods. The highest mortality of larvae was reported in dust spray method where mortality was increased from 58% to 85% at 24 h and 72 h of treatment using 1 mg/cm$^2$ concentration. This mortality rate could be further increased with increasing concentrations and duration of exposure. The death of feeding larvae occurred due to desiccation, abrasion of body walls and spiracle blockage[12].

**Single walled carbonnanotube (SWCNT)**

It was produced by catalytic vapour deposition (CVD) and reported to exhibit mortality in *P. xylostella* larvae only at higher concentrations such as 138.24 µg/mL concentration, larval feeding reduced upto 10% and the morphology of the larvae also demonstrated darker colour which implies that SWCNT has been deposited in the larval gut. As concentration of carbon nanotubes increased there was significant reduction in pupation rate, as well as fecundity (egg/adult) rate also declined upto 45% with increasing SWCNT concentrations. The SWCNT feeding larvae showed no or minor increase in larval size with increasing food consumption[13].

**Titanium dioxide nanoparticles ($TiO_2$NPs)**

Titanium dioxide nanoparticles ($TiO_2$) in its ionic form has been considered as active compound and proved as available alternative to the synthetic pesticidal control in the management of pest in the crop fields. Titanium dioxide $TiO_2$-NPs synthesised by sol-gel method[14]and dose dependant response was obtained through regression analysis for the bioassay of titanium dioxide ($TiO_2$) while tested on diamondback moth larvae with different concentration and different time period interval, respectively. The cumulative mortality was nearly 50%. The above result was supported by Kookana *et al*. (2014)[15] who suggested that nanomaterials could be an alternative approach against synthetic insecticides for insect pest management.

**Emamectin benzoate nanoformulations**

This approach utilizes colloidal form of delivery system which effectively controls pesticides delivery to increase photostability and efficacy of semi-synthetic and natural pesticides. The prepared emanectin benzoate nanoformulations involved polymeric nanocapsules and nanosilica of two types, mesoporous and silicon dioxide which worked as carriers for emamectin benzoate. This type of combination displayed high encapsulation efficacy of upto 70-90% in different nanocapsules. The efficacy of nanocapsules against *P. xylostella* was found to be EB+SNPs had more effective in comparison to other EB+NFs and EB placebo. The $LC_{50}$ values for EB+SNPs, EB+MCM-48, EB+PNC and EB placebo would be 0.18, 4.03, 8.49 and 11.06 mg, respectively[12]. The enhanced efficacy of nano-capsulations was due to smaller particle size, large surface area and their mobility increment ratios which lead to the high penetrance ratio in the larval body causing higher mortality at smaller dosage.

**Silica nanoparticles (SiNPs)**

Because of the known properties of the controlled release, the major attention had been given to the silica nanoparticles for smart delivery system potentials. For the preparation of florescent mesoporous silica NPs with embedding of Carbon dots (FL-$SiO_2$ NPs), silica precursors had been used *via* tetraethoxysilane (TEOS) involvement and structure-directing agents as Cetyltrimethylammonium bromide (CTAB). The leaf-dipping method was used for the detection of insecticidal activity against insect larvae. In this experimental setup the control has been Indoxacarb technical concentration (TC) and various gradients of descending order 25, 12.5, 6.25, 3.12, and 1.56 mg/L had been tested. The mortality of larvae after 48 h of treatment was 90% for IN@FL-$SiO_2$ NPs and for control data 76% at 25 mg/mL of higher concentration. The FL-$SiO_2$ NPs presence had been observed at the midgut of *P. xylostella* using confocal laser scanning microscopy (CLSM)[16]. The higher mortality of *P. xylostella* larvae in the treatment was observed due to decreased enzymatic activities which could imply the nano-based delivery system of insecticides for the decreasing or delaying the effect of insecticide resistance. This method could be included for resistance selection of pest to IN@FL-$SiO_2$ NPs and for identification of resistant strains of diamondback moth using bioactive and biochemical assay[17].

The effect of microsizedchlorfenapyr (MCSC) and nanosized chlorfenapyr (NCSC) against *P. xylostella* was analysed[18] and found that NCSC caused higher mortality in the larvae and also required half dose of treatment as compared to MCSE. The higher larval mortality was observed from first day and subsequent days that confirmed the reduced amount of pesticides as well as less time interval through applying nano silicons. The enhanced activity had been reported due to improvement in biological efficacy of chlorfenapyrl-loaded silica as well as silica itself provided insecticidal activity[18]. Kaziem *et al*. (2018)[19]

reported that avermectin (AVM) controlled released using α-Amylase carriers had been prepared using lair mesoporous silica with α-CD as capping molecule. The AVM-CRF biological tests were conducted on *P. xylostella* larvae through uncapping of α-CD enzyme which later on release AVM causing larval deaths. The combination had been able to keep 0.6 mg/L AVM to be in bull form for $14^{th}$ of the subsequent days showing 83.33% mortality of the insect larvae.

**Biofabricationof nanopesticides**

A lot of techniques have been designed using variety of physical, chemical methods for synthesis of nanomaterials. This field had shown different properties of the same chemical in the bulk scale and in the nano scale. In the nanoscale, because of larger surface area the atoms provided more chemical reactions and the time of exposure also increases to initiate any reaction. As we have seen in the above context, with the help of nanotechnology variety of new pesticides, insecticides and insect repellents had been prepared. Some chemicals used during the synthesis of these nanoparticles in polyol method, microemulsion, electrochemical decomposition, and thermal decomposition are found to be very toxic for the environment as well as for human beings. To overcome this negative effect of the NPs we are on the brink to developed methods of preparation involving green synthesis approach. The new emerging arena of green synthesis involves plants metabolites for the reducing agents, oxidising agents, and so on. These plant products used for biofabrication are cost-effective, environment friendly and with minimum adverse effects of the human beings[4]. The entrapment of pesticides inside polymers increases the stability of the compound and causes slow release which finally decreases the dose of the same compound and also decreased side effects. There are still a lot of unexplored fields available to be worked on. The list given below provides the successful biosynthesis of NPs used in the agricultural field to control the insect pest *P. xylostella* (Table 1).

**Biopolymer based silver nanoparticles (AgNPs)**

Biofabrication of silver nanoparticles (AgNPs) have been considered as highly reliable method[21] to use it as an alternative way to manage the population of *P. xylostella*. Biosynthesized silver nanoparticles (AgNPs) using neem plant demonstrated highest mortality of 76% at a concentration of 2.4 mg/mL and minimum mortality of 28% at the concentration of 0.15 mg/mL against the $3^{rd}$ instar larva of diamondback moth for the 24 h of time exposure. Proceeding of the same experiment for 48 h showed that 80% and 32% of mortality for the maximum and minimum concentrations, respectively. The result for 72 h found to be 92% and 36% of the mortality for the higher and lower concentrations, respectively[1].

When clove (*Syzygium aromaticum*) was used for biofabrication of AgNPs and its effect was evaluated on larvae of diamond back moth for 24 h, larval

Table 1- Application of various nanoparticles for the management of *Plutella xylostella*

| Nanoparticles | Synthesis | $LC_{50}$ | Mode of action | References |
|---|---|---|---|---|
| Nano-silica | sugarcane | 30.03 µg | - | Kannan *et al*. 2017 |
| Silicon dioxide | Sol-gel technique | 0.99 mg/L | Dessication, abrasion of body walls | Shoaib *et al*. 2018 |
| SWCNT | Catalytic vapour deposition | 138.28 µg/ mL | Stored in the gut with zero metabolism and bind with actin filament centromere | Taiaba Afrin 2017 |
| Titanium dioxide | Sol-gel method | Cumulative mortality | _ | Kokkan *et al*. 2014 |
| Emamectin benzoate nanoformulation | Colloidal form for delivery | 0.18 mg | Penetration into the larval body | Shoaib *et al*. 2018 |
| Silica nanoparticles AVM-CRF | Chemical method | 0.6 mg/L | Slower release of pesticide AVM | Kaziem *et al*. 2018 |
| Silver nanoparticles | *H. musciformis* | 24.5 ppm | Decreases fecundity and longevity | Roni *et al*. 2014 |
| Neem oil | Neem extract biosynthesis | 0.94 mg/ mL | Slower release of neem oil | Forim *et al*. 2013 |
| Bt-based nanopesticides | Top-down approach homogenization using water | - | Crystal toxin in the midgut alkaline medium | Kim and Joe, 2012 |

mortality of 76% at the dose of 2.8 mg/mL and 24% for the dose of 0.175 mg/mL was observed. After 48 h, the mortality was further increased to84% and 32%, respectively. After the treatment for 72 h the mortality was88% and 36% for the higher dose and lower dose where the highest dose mortality had been 80% and 88% whereas the lowest dose of mortality of 32% and 36%[1]. Synthesis of AgNPs using bitter gourd (*Momordica charantia*) added further toxicity when tested against *P. xylostella* larva for the maximum concentration of 3 mg/mL and minimum concentration of 0.1875 mg/mL showed 72% and 24% of mortality, respectively. After 72 h of treatment, the reported mortality for the same concentration found to be 84% and 36%, respectively[1].

Biofabrication of AgNPs using Datura at a concentration of 2 mg/mL exhibited highest mortality of 72% and for the minimum dose of 0.125 mg/mL reported 28% of effects for only 24 h of time duration. After treatment of 48 h, larval mortality increased upto 80% and 32%for higher and lower concentration, respectively, and the report of 72 h recorded values of 84% and 40% for the former and later, respectively[1]. AgNPs biosynthesized using *H. musciformis* sea weed leaf extract exhibited larvicidal and pupicidal toxicity at low concentration against *P. xylostella* and also decreased fecundity and longevity of adults of diamondback moth. The $LC_{50}$ value of AgNPs for $L_1$ and pupae had been reported as 24.5 ppm and 38.23 ppm for *P. xylostella*[20].

Silver nanoparticles biosynthesis using bakain (*Melia azedarach*) was found to exhibit toxicity against insect larvae because of the toxic effect of the plant itself[1].When these Ag-NPs were tested against *P. xylostella* for 24 h showed the maximum mortality of around 72% for the dose of 4 mg/mL and minimum mortality rate of around 28% for the dose of 0.25 mg/mL when the experiment proceeded for 72 h at the dose of 0.1875 mg/mL showed 44% of larval mortality and the maximum dose of the same experiment would be recorded at 3 mg/mL of 80% mortality implies killing of 20 out of 25 larvae in the experimental apparatus[1]. AgNPs biofabrication using garlic demonstrated highest mortality of *P. xylostella* larvae of 72% after 24 h of treatment[21]. When this preceded further for 24 h on the subsequent day exhibited 80% of the total mortality. When further proceeding upto 72 h, the larval mortality was recorded to be 84%[21]. It had also been reported that garlic also exhibited insecticidal activity against *P. xylostella* larvae[22].

**Nanocapsules**

Biofabrication of nanocapsules were performed using *Azadiracta indica* oil and neem extracts involving polymer and emulsion had been tested against *P. xylostella* larvae. Kale leaves dipped in different concentrations of treatment were provided as food for the larvae. The result exhibited 100% mortality in first instar larvae on the 9$^{th}$ day of treatment as well as powdered NPs caused only 91.7% mortality[23]. Same results were also demonstrated by Torres *et al*. (2006)[24] where neem extract caused 100% larval mortality. The different mechanisms of action involved in the solution phase dipped with kale leaves included solvent phases causes active compound release earlier whereas the powdered form caused later release of active components, greatly dependent on the colloidal silica hydration. This provided a bio-sustainable process involved no harmful chemicals.

**Bt-based nanopesticides**

This category includes Bt size decreased via Bt technical powders using top-down method as well as Bt-homogenization through water as an emerging method. Smaller sized Bt-homogenized particles caused increased DBM larval mortality. The unhomogenized powder caused larval mortality of 27.5% within 2 days of treatment which increased upto 72.6% in day three whereas homogenized Bt-nanopesticides exhibited 78.3% even on day two and 100% mortality on day three[25]. The mechanism of action involved in nanoparticles of Bt enhanced the solubility of the crystal toxin in the midgut alkaline media which caused more production of small size toxins that actively binds with the receptors and ultimately caused rapid gut paralysis followed by larval death.

**Conclusion**

The nanotechnology has provided promising methods for the successful implementation in the field of agriculture for the development of nanopesticides. The green synthesis approach uses plants or plant products which significantly decreased our reliance over the use of toxic chemicals. The biofabrication of silver using various plant materials such as garlic, cloves, bitter gourd, bakain, which is more effective than its simple NPs synthesized using chemicals. The plants products itself also have pesticidal activity which further improves the efficacy of nanopesticides and subsequently decreases the dosage of that pesticides. So, it has been concluded that the biosynthesis using green approach would be a significant step towards the development of nanotechnology with minimum or no less at all.

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**Conflict of interest**

All authors declare no conflict of interest.

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