

# Nano bio pesticide to constraint plant destructive pests

## Abstract

Pest affects plants in nature worldwide, leading to excess use of mineral fertilizers and toxic pesticides, effecting environments, plants, animal and causes serious health problems to farmers. The indiscriminate use of bio pesticide has led pest resistance, reduce soil fertility and finally there is an emergence of novel product to intercept the pest situation. Nanoscience a new discipline have great deal of application in various fields and may also be useful in agrochemical and plant protection area to control pest to considerable extent. Until now, nanoparticles were used in formulation of nano based pesticides and insecticides, encapsulated nanoparticles, nanoparticle-mediated gene or DNA transfer in plants and bio sensors for remote sensing for precision farming. The nanopesticides of biological origin named as bio- nanopesticide could be fabricated using any metal such as Ag, Cu, SiO<sub>2</sub>, ZnO with broad-spectrum pest protection efficiency. However, extensive research and spectrum of various field studies are specially required to develop understanding of interaction between nanoparticles, microorganisms, soil, plants and humans. In present paper, a critical analysis is addressed in a problem of pest in field and usage of conventional solution and nano bio pesticides for applications in crop protection systems.

**Keywords:** Silver nanoparticles, Pest management, Nano Biopesticides, *Helicoverpa armigera*, Crop productivity

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## Introduction

Insects-pest is animal populations, which occurred in every possible environment with a varied number of species. The several insects are vectors of different diseases, and cause damages to crop plants. These have been affecting economy and the yield of crop plant and at national and international market. The crop yield losses caused by insects in agriculture, several chemicals have been applied to control them.<sup>1</sup> Insect- pests are one of the leading causes that affect agriculture productivity resulting in billion dollars loss per annum.<sup>2</sup> The two main forms i.e. larva and adult have been most lethal for most of the crop plants. For example, the deadly pest *Helicoverpa armigera* cause damage in the field and horticulture crops around the globe.<sup>3</sup> It has been major prevalence affecting in Asia, Europe, Africa, Oceania and South America.<sup>3</sup> The major crop plants that are affected by *Helicoverpa armigera* are Chickpea, Corn, Cotton.<sup>4</sup> Peanut, Pigeon pea, Sorghum and Tomato. *H. armigera* has been effecting on over 180 cultivated hosts and 45 families of wild plant species.<sup>3</sup> The cotton yield is low due to 150 different pest attack at various life stages.<sup>5</sup> This has made an emergence for a researcher to compile the traditional and advance techniques to overcome various pest threats around the world.

The insect-host plant interaction leads to activation of plants immune system and there is cross talk between various pathways for production of both primary and secondary metabolites. The cell wall acts as barrier to insect however, becomes crossable for lethal insects leads to final destruction of plant. The secondary metabolites produced acts as specific defensive systems that are activated upon insect interaction. The phenylpropanoid pathway metabolites have extensive role for formation of various metabolites with definite characteristic. The plant secondary metabolites helps plant in several ways such as (i) prevention of herbivore and pathogen attack, attraction of pollinators and symbionts, and plant-plant communication.<sup>6</sup> Thus secondary metabolites based biopesticides are the natural source that could be targeted to get rid of pest and are ecofriendly. These biopesticides are organic, low-risk, environmentally friendly as

compare to the synthetic pesticides. Thus, the exploration of plants that having potential against insect pest is the chief for controlling their population and to save agriculture losses.

The plant such as weed plant that having no importance in agriculture could be dissects to identify many useful secondary metabolites that can be effective against pests. There are some plants with inherent capacity to deal pest, their extract shows different insect resistant compounds such as for example camphor, limonene and b-caryophyllene from *Ocimum kilimandscharicum*.<sup>7</sup> The neem extract is pioneered for characteristic of larvicidal and pesticidal activity.<sup>8</sup> The weed plant *Argemone maxicana*.<sup>9</sup> *Calotropis procera*.<sup>10</sup> *Datura*, *Catharanthus roseus* contents phytochemicals and secondary metabolites essential to minimize insect population. The need to formulate the biopesticide with efficient insect killing property is attempted by mixing different secondary metabolites in various ratios. However the constant use of the best sale biopesticide has put itself in danger for resistance in pest. It has allowed scientists and researchers all over the world to assemble the efficient secondary metabolites against insect-pest. The other alternative hidden in nature against pest needs to open up with the existing technologies. The greener method to avoid pollution and safer use with minimum toxicity for animals and plants is the best alternative. The Green chemistry topic that uses the green natural material as starting source to formulate the nano size particle capped with the pest resistant secondary metabolites could be possible solution to defect pest in farms.

In respect to above scenario, the current paper discusses the general demand for biopesticides, chemical, biological ways to dealt pest and the latest nano- biopesticides that can be formed using different plant extract complex with silver nano particles and their essays against pest- insect are communicated.

## Discussion

There are many varied types of pesticides is used to treat effective against specific pests. The term "cide" comes from the Latin word

“to kill”.<sup>11</sup> There are thousands of private pesticides manufacture, formulator and producer with some National companies in different countries. The Pesticide products are usually consists of active ingredients and other ingredients. The active ingredients is main entity that eliminates pest, in contrast, the other ingredients assist in several ways such as attracting the pest, spreading the active ingredients around, and/or reducing drift.<sup>11</sup> The Pesticides are classified according to their targets as shown in Table 1. In current paper, the insecticides are the main concern that destroying thousands of plant species.

**Table 1** Show classification of Pesticide according to their target

Sr no	Pesticide Type	Target Pest
1	Nematicides	Nematodes (roundworms)
2	Molluscicides	Slugs and snails
3	Insecticides	Insects
4	Acaricides (or miticides)	Fleas, ticks and mites
5	Piscicides	Fish
6	Avicides	Birds
7	Rodenticides	Rodents
8	Bactericides	Bacteria
9	Algicides	Algae
10	Fungicides	Fungi
11	Herbicides	Plants

## Global demand of biopesticides

In the contemporary world the main focus is to design, formulate the ecofriendly pesticide that produced for a minimum amount to the investment with the efficiency of high potential. The eco-friendly bio pesticide's formulation and their demand as product consumption by farmers were elevated. The nations of a world put constant effort for development of efficient bio pesticides. It was observed that the global bio pesticide and synthetic pesticide market grew from \$54.8 to \$61.8 billion in 2013-2014 and estimated to expand to \$83.7 billion by 2019.<sup>12</sup> Furthermore, the global sale for bio pesticide is estimated to reach a USD 6.9 billion by 2019. The scenario in Asia-Pacific region ergs for 20% demand for bio pesticides, however, the North America dominated by a requirement of 40% of market demand. There are many countries that rely on bio pesticides for pest managements in agricultural biotechnology to provide food security.<sup>13</sup> Thus the sale of bio pesticides has been increased as compared to chemical's pesticides.

**Chemical-based pest management (traditional trend):** The most common chemicals pesticides are available in local pest store, online source such as Amazon and there are several manufacture, dealer and exporter. In early days Sulphur element was used to make several chemicals products such as sulfuric acid, phosphate fertilizers, fungicides and insecticides. Apart from sulphure the general active chemicals ingredients are abamectin, cyfluthrin, fipronil, permethrin, bifenthrin, hydramethylnon, pyrethrum and boricacid. The chemical based pests are classified on basis of their chemical structure like organophosphates, carbamates, organochlorines, pyrethroids and neonicotinoids. Table 2 shows some of the important chemicals that used as insecticides with their effect on animal, insect, plant and humans. There are many insecticides available at the market some of them are acephate, azadirachtin, bioresmethrin, carbaryl, carbaryl, dichlorvos, fenitrothion, malathion, pirimiphos methyl, pyrethrum and quinalphos. The management of Pest has elevated the use of variety of chemical insecticide, pesticides and synthetic insecticide i.e. pyrethroids. The continuous exposure of synthetic pyrethroids has led the insect to escape through resistance.<sup>14</sup> Thus the traditional chemicals based pesticides are failed to manage a pest on crop plants. These have

an emergence the use of alternative mode for plant protection i.e the use of biological plant derived natural compounds, phytochemicals and secondary metabolites. The chemicals based nanoparticles are synthesized and applied to evaluate their efficiency against larvae. The nanoparticles of novaluron, a water-insoluble insecticide was prepared which consists of nanoparticles sized 30-100 nm that shows toxicity in vivo experiments with Egyptian cotton leafworm *Spodoptera littoralis* larvae. However, the byproducts remains after experiments were hazardous chemicals thus the chemicals are not the choice for nanoparticles development in agriculture chemicals sector.<sup>15</sup>

**Table 2** The chemical structure and effect on insects humans and other animals

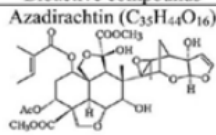
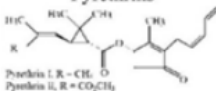
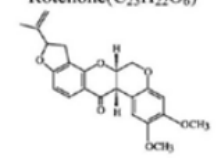
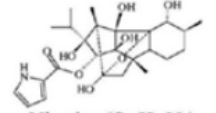
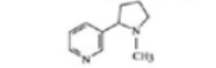
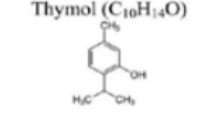
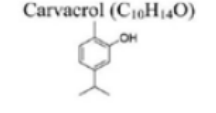
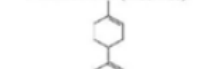
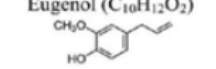
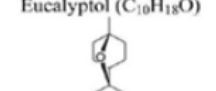
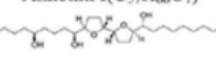
Sr no	Name of Chemicals	Effect	Activity
1	Organophosphates	Insect, humans and other animals	Kill insects by interfering with brains and nervous systems.
2	Carbamates	They break down quicker, less dangerous to humans	Kill insects by interfering with brains and nervous systems.
3	Organochlorines Dichlorodiphenyl trichloroethane	Found in soil, in the bodies of animals and humans. Causing the developing babies to die.	Persistent pesticide, not breakdown easily, disrupts human endocrine systems, damage genes and risk of cancer
4	Pyrethroids	Less effect to human	Feelings of numbness, itching, burning, stinging, tingling.
5	Neonicotinoids	Neonicotinoids are derived from nicotine, a plant chemical, effects many species & mammals.	Interfere with a pathway of insect nervous system and role in honeybee colony collapse disorder

**Biological-based pest management (old trend):** The plant derived phytochemicals have been used as biopesticide with an advantage of non toxic to animals and human. The plants secondary metabolites such as alkaloids, terpenoids, flavonoids, phenol, polyphenons, glycosides and tannins have varied bioactivity against harmful insects. These secondary metabolites based bio pesticide has managed the pest *Helicoverpa armigera* to certain extent.<sup>16</sup> Some other common examples that are biological control are *Anopheles subpictus*, *Bacillus thuringiensis*, *Bacillus thuringiensis serovar kurstaki*, *Beauveria bassiana* and *Culex quinquefasciatus*.<sup>17</sup> There are several research publications indicating the optimistic effect of medicinal plant extracts, powder, against pests such as Mosquito and *Helicoverpa armigera* etc. Large number of curative plants that have a characteristic of larvicidal and pesticidal activity such as neem extract.<sup>8</sup> *Acorus calamus*, *Annona squamosa*, *Vitex negundo*.<sup>18</sup> *Gnidia glauca*, *Toddalia asiatica*.<sup>19</sup> and *Argimone maxicana*.<sup>9</sup> *Calotropis procera*.<sup>10</sup> The work of.<sup>20</sup> confirms the percent infestation reduction is highest in neem seed kernel extract (30.08%) followed by tobacco leaf extract (28.68%). The aqueous extract of neem seed kernel (NKAE) was tested in laboratory condition to evaluate its antifeedant, ovicidal, nymphal duration and hatching performance on tea mosquito bug (*Helopeltis theivora*).<sup>21</sup> The Table 3 displays some sources of botanical pesticides, active compound and their mode of action. The plant parts such as fruit stem, root, fruit and leaves have been molded into extract and their efficiency has been checked against insect-pest. The.<sup>22</sup> tested aqueous bark extract of *Ficus racemosa* was tested against fourth instar larvae of filariasis vector, *Culex quinquefasciatus* and Japanese encephalitis vectors, *Culex gelidus*. The bark extract of *F. racemosa* LC50 and r2 values were calculated and maximum efficacy against the larvae of *Cx. quinquefasciatus* and *Cx. gelidus* (LC50=67.72 and 63.70 mg/L; r2=0.995 and 0.985). The use of plant parts extract is always an upper choice from ecological point of view. The extract contents various

bioactive metabolites that possessed pesticidal property. Some of the sources of plant carrying pesticidal property along with their mode of action are given in Table 3. However, indiscriminate application of biological pesticide formed using parts of plants would come out

with the resistance and there will be an emergence of novel product to tackle the pest situation. Thus another scientific alternative need to explore for minimizing Pest effect on crop plants.

**Table 3** Some sources of botanical pesticides, active compound and their mode of action. The data have been retrieved from.<sup>23</sup>

Sources	Bioactive compounds	Function	Mode of action	Properties
Seed and leaf extracts of <i>Azadirachta indica</i>	 Azadirachtin (C <sub>35</sub> H <sub>44</sub> O <sub>16</sub> )	Insecticide & fungicide	Blocks the synthesis and release of moulting hormones (ecdysone). Disrupts the normal mating behaviour and results in reduced fecundity. Anti-feedant / repellent effect on many insects.	Photo-degradable Half-life 20 h Acute oral LD <sub>50</sub> to rat is >5000 mg kg <sup>-1</sup>
Dried flowers of <i>Chrysanthemum cinerariaefolium</i>	 Pyrethrins Pyrethrin I, R = CH <sub>3</sub> Pyrethrin II, R = CO <sub>2</sub> CH <sub>3</sub>	Insecticide & acaricide	Disrupts the sodium and potassium ion exchange process in nerve axons. Rapid knockdown effect on flying insects.	Photo-degradable Acute oral LD <sub>50</sub> to rat is 350-2000 mg kg <sup>-1</sup> (depends on purity)
Roots and rhizome extracts of <i>Derris</i> sp., <i>Lonchocarpus</i> sp. & <i>Tephrosia</i> sp.	 Rotenone (C <sub>23</sub> H <sub>22</sub> O <sub>6</sub> )	Insecticide, acaricide & piscicide	Inhibits cellular respiration (at site I) within electron transport chain and prevents energy production.	Highly toxic to fish Acute oral LD <sub>50</sub> to rat is 132 mg kg <sup>-1</sup>
Stem extracts of <i>Ryania speciosa</i>	 Ryanodine (C <sub>25</sub> H <sub>35</sub> NO <sub>9</sub> )	Insecticide	Affects muscles by binding to the calcium channels in the sarcoplasmic reticulum.	More effective on selected species Acute oral LD <sub>50</sub> to rat is 1200 mg kg <sup>-1</sup>
Leaf extracts of <i>Nicotiana glauca</i>	 Nicotine (C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> )	Insecticide	Causes continuous uncontrolled nerve firing by binding with acetylcholine receptors at nerve synapses. Act as fumigant against sucking pests	More effective on selected species Acute oral LD <sub>50</sub> to rat is 50 mg kg <sup>-1</sup> Dermal adsorption in human
Essential oil of <i>Thymus vulgaris</i>	 Thymol (C <sub>10</sub> H <sub>14</sub> O)	Fungicide, bactericide & insecticide	Inhibits bacterial growth, lactate production and decreases cellular glucose uptake. Alters the hyphal morphology and causes hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall.	Minimal potential toxicity and poses minimal risk. Degrades rapidly (DT <sub>50</sub> 16 days in water, 5 days in soil).
Essential oil of <i>Origanum vulgare</i> , <i>Thymus</i> sp., <i>Origanum majorana</i> ,	 Carvacrol (C <sub>10</sub> H <sub>14</sub> O)	Bactericide	Disrupts cell membrane of bacteria, e.g. <i>Pseudomonas aeruginosa</i> . Inhibits the growth of several bacteria strains, e.g. <i>Escherichia coli</i> and <i>Bacillus cereus</i>	In rats, carvacrol is metabolized and excreted within 24 h.
Fruit extracts of <i>Citrus</i> sp.	 Limonene (C <sub>10</sub> H <sub>16</sub> )	Insecticide	Used as repellent	Non-toxic to humans, birds and animals
Bud and leaf extracted essential oils of <i>Syzygium aromaticum</i> ,	 Eugenol (C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> )	Insecticide	Used in bait to attract and collect insects	Causes hepatotoxicity in humans
Essential oil of <i>Eucalyptus globulus</i>	 Eucalyptol (C <sub>10</sub> H <sub>18</sub> O)	Insecticide	Used as repellent Used in bait to attract and collect insects	Acute oral LD <sub>50</sub> to rat is 2480 mg kg <sup>-1</sup>
Seed extracts of <i>Annona</i> sp.	 Annonin I (C <sub>37</sub> H <sub>66</sub> O <sub>7</sub> )	Insecticide	Inhibitory effect on the NADH-cytochrome c-reductase and complex I of insect mitochondria	In pure form is toxic to mammals (LD <sub>50</sub> is <20 mg kg <sup>-1</sup> )

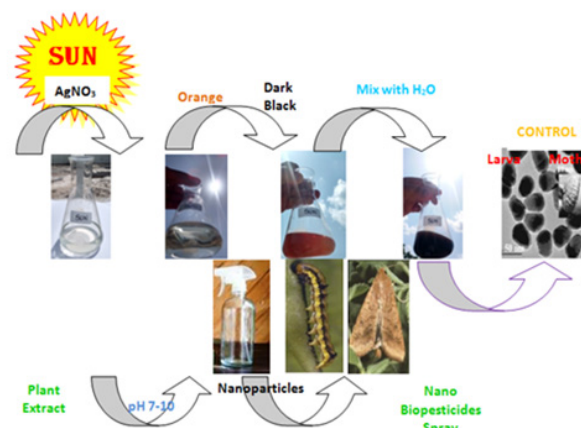


**Nano-based pest management (new trend):** Nanotechnology has been proposed to considerable extent and have been applied in numerous formulation to create many new products with a wide range of applications in several fields such as textile, geo sensing technology, paper, food, fertilizers, pesticides, plant protection, nutrition paint, food bio fuel, biomass, biocomposites and agrochemical industries.<sup>24</sup> Thus, nano based innovative nanopesticides such as Ag, Cu, SiO<sub>2</sub>, ZnO and nanoformulations show better broad-spectrum pest protection efficiency, reducing water, soils and environmental pollution in comparison with conventional pesticides.<sup>25</sup> The Zn is an essential nutrients for plants and is less available in soil this makes Zn metal as an important target for development of Zn nanoparticles for dealing with the pest and beneficial for plants. ZnO NP are of less cost and safe. They are applicable in various field such as anti- cancer, anti-diabetic, antibacterial, antifungal and agricultural properties, agrochemical industries. The other potential option could be silver. The silver (Ag) is used in the field of the biological system, living organisms, medicine, plant management, pest control and agricultural aspects with better efficiency and activity are the preferred target of the green method related to antibacterial, microbial, fungal, larvicidal, pesticidal, anti-inflammatory, antiplatelet activity, anti-angiogenesis and anti-viral activity.<sup>8,25-27</sup> The antifungal activity of ZnO nanoparticles against plant pathogen *Fusarium graminearum* have been demonstrated by.<sup>28</sup> The metal nanoparticles has an advantage of stability, slow kinetics which can be scale up for large quantity, performed at room temperature and generation of eco environmental byproducts.<sup>29</sup> Thus nanoformulation of bio pesticide could be the best possible alternative for development of pest-control weapons for harmful insect. Figure 1 shows the complete process of silver nanoparticles synthesis using *Alo vera* extract and AgNO<sub>3</sub> under SUN conditions and its spray application to control insect-pest *H. armigera*. The synthesized bio nanoparticles (nanocorns shape) were mixed with water in definite quantity and filled in spray bottle, application on pest-insect *H. armigera*. Larvicidal activity of synthesized silver nanoparticles (AgNPs) using aqueous extract from *Eclipta prostrata* have been utilized to control the mosquito.<sup>30</sup> The fourth instar larvae of filariasis vector, *Culex quinquefasciatus* say and malaria vector, *Anopheles subpictus* Grassi (Diptera: Culicidae) were subjected to evaluation. The synthesized AgNP of 35–60nm in size shows he maximum efficacy in crude aqueous, and synthesized AgNPs against *C. quinquefasciatus* (LC50 = 27.49 and 4.56 mg/L; LC90 = 70.38 and 13.14 mg/L), and against *A. subpictus* (LC50 = 27.85 and 5.14 mg/L; LC90 = 71.45 and 25.68 mg/L) respectively.<sup>30</sup> The work of.<sup>31</sup> shows that the leaves aqueous extracts of *E. prostrata* have the potential to be used as an ideal eco-friendly approach for the control of the *S. oryzae*. The LD50 values of aqueous extract, AgNO<sub>3</sub> solution and synthesized Ag NPs were 213.32, 247.90, 44.69 mg/kg -1; LD90=1648.08, 2675.13, 168.28 mg/kg -1, respectively. The Larvicidal efficacy of *Catharanthus roseus* Linn leaf extract have been positive *Anopheles stephensi* Liston.<sup>32</sup> The pest *H. armigera* specific work of.<sup>33</sup> used leaf extracts of Peepal tree, *Ficus religiosa* (FR) and banyan tree, *Ficus benghalensis* (FB) for fabrication of AgNPs to modulate the function of gut protease activity in *H. armigera*. They confirmed bioassay of AgNPs with FR (50% concentration) and FB (70% concentration) capable for reduction in larval weight and survival rate of *H. armigera*. However the pure larvicidal compounds integrated with silver will always show better results than the mixture of secondary metabolites. This strategy needs to explore with reference to plant extract containing larvicidal activity.

### Possible nano bio-pesticides structures

The plant protection is being the busiest area for researcher for the

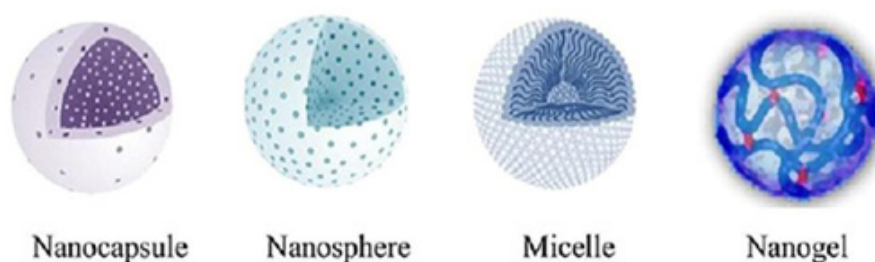
formulation of active solution to overcome the synthetic commercial pesticides. The traditional methods mixed with nanoscience with improved efficiency such as better solubility, slower releasing, and avoidance of premature degradation.<sup>23</sup> The biological compounds acts as capping and reducing agents to silver salt and formation of stable nanoparticles takes place. Figure 2 shows the capping of repellent compound Aloin from *Alo vera* on formed silver nano particles. These nano biopsticides or nanomaterials could be used as a pesticide directly or indirectly as vector to inherent important characters such as stiffness, permeability, crystallinity, thermalstability, and biodegradability. These properties are advantageous over chemical or commonly used pesticides. The nanocarrier materials with definite pesticides secondary metabolites can cause indigestion in insects. They can break down the water protection barrier, resulting in desiccation and death of insect. The nano size helps to act efficiently to insect with minimum amount of nano bio-pesticides. The nano material also helps to protect the active secondary metabolites and assist in controlled release of bioactive compound to act on insect.<sup>23</sup> These are the possible ways in which nano bio-pesticides can be formed using plant pesticidal secondary metabolites and application of it in form of spray will ultimately help in pest removal and resistance. Figure 3 shows the possible nanostructure used for formulation of nano bio-pesticides using plant secondary metabolites.



**Figure 1** The complete process of silver nanoparticles synthesis using plant extract and AgNO<sub>3</sub> under SUN conditions, From left to right; The leaf extract (Aloe vera) green color used for reduction of silver salt AgNO<sub>3</sub>, the desired pH of mixture is adjusted to 7-10, the nanocorn nanoparticles applied in form of spray to pest-insect, picture was taken from.<sup>26</sup>



**Figure 2** The capping of repellent compound Aloin from *Alo vera* on formed silver nano particles. This image retrieved from.<sup>34</sup>



**Figure 3** The possible nanostructure used for formulation of nano bio-pesticides, Pictures retrieved from.<sup>23</sup>

### Required qualify for selection as nano bio-pesticides

In order to be safe, easy to prepare, low cost and effective pesticides to diminished the plant pest, the important points that needs to be consider are first, it must be easy for preparation, it must be effective at economic rates, could be targated against specific pest, may be effective against a wide variety of insect pests, it must be safer to all living beings and environments, it must be non toxic to farmers, it must not content any harmful sub substance, must be approved by health authorities, must not get accumulate in food chain, it must not give rise to unacceptable residues, legal lethal dose concentration must be framed, it must not effect the quality of food, flavor, fragrances and texture, It must be acceptable in international market, it must not be flammable, explosive or corrosive, it must be easily applicable.

### Need of research

Although there have been numerous studies of the toxicity effects of nanoparticles on bacteria, fungi, and animal pathogens. Little research has been carried out to investigate the toxicity effect of nanoparticles on insect's pest such as *Amsacta moorei* Butle, *Episomus lacerta* F, *Brachytrypes portentosus* Licht, *Chrotogonus* sp, *Gryllulus domesticus* Linn, *Helicoverpa armigera*, *Laphygma exigua* kb, *Mylocerus maculosus* Desbr, *Pempheres affinis* Fst *Platyedra gossypiella* Saund, *Ragmus morosus* Ball, *Termites*, *Zeuzera coffeae* Nietn.<sup>35</sup> The extensive funding and direction in this area could be targeted to come up with the great findings for plant protection. The plant species containing secondary metabolites that are insect repellents must be identified and complexes with silver or other nano particles. These biologically synthesized nanoparticles effect on different pest- insect needs to clarify. This will conclude the plant species specific resistance for particular insect.

### Critical points for nano-biopesticides

There is a great concern regarding the nano bio pesticides material, which has been potential to exert hazardous effects on soil, human and environment. The positive effect of nano-pesticide may have some negative effects, which need to be resolved by critical research. The interaction of nano based pesticides with soil, and chemical interaction's soil organic matter are other important aspects. Apart from nanoparticles the prime importance to acknowledge the particular plant species carrying peculiar photochemical may play a crucial role for nanoparticles formations. The plant inherited with pesticide phytochemical and secondary metabolites influences the future formed nanoparticles. There are some plants extract that have been exploited for insecticidals activity such as *Catharanthus roseus* L, *Datura metal* L, *Cardiospermum halicacabum* L, *Argemone mexicana* L, *Calotropis procera* L, *Azadirachta indica* L, *Oscimum species* wild, *Oscimum sanctum* L, *Oscimum canum* L and *Citrus sinensis* L.

### In-vitro bioassay for Nano-biopesticides against pest control

The lab based assays are important for potential determination of synthesized silver nanoparticles, which is called as an *in-vitro* assay. The possible foremost target for evaluation of synthesized SNP could be the food the pest prefers, the life stages of pest such as larvae, pupa and adult moth. Thus the antifeedant, larvicidal and cytotoxic activities of synthesized Nano-biopesticide fabricated from aqueous leaf extract of plants were of principal concern. The insect may be collected from any standard Indian Council of Agriculture Research (ICAR) Institute and instar larva could be maintained in lab. The antifeedent, pupicidal, insecticidal activity can be tested for establishment of potential nano based product for agriculture plant protection.

**Antifeedent activity:** The plant extracts possessing antifeedant and insecticidals activities against several insect-pests has been demonstrated. The SNP synthesized using leaf aqueous extract of *Manilkara zapota* shows feeding deterrent activity against *Musca domestica*.<sup>36</sup> The leaf disc no choice method is usually employed.<sup>37</sup> In this, a fresh leaf disc of around 4 cm in diameter is dipped in varied concentrations of synthesized SNP extracts. The water can be taken as negative control and the leaf extracts as positive control. The petri dish is covered with hydrated filter paper, and one third instar larva may introduce into each petri dish. The consumption of leaf area by the treated and control larvae after 24 h was recorded using leaf area meter. The Leaf area, eaten by larvae in treatment was corrected from the negative control. The antifeedant activity could be measured using the formula given in Figure 4. The potential antifeedant (92.40 %) activities against *H. armigera* with LC<sub>50</sub> values of 365.72 was recorded by.<sup>38</sup>

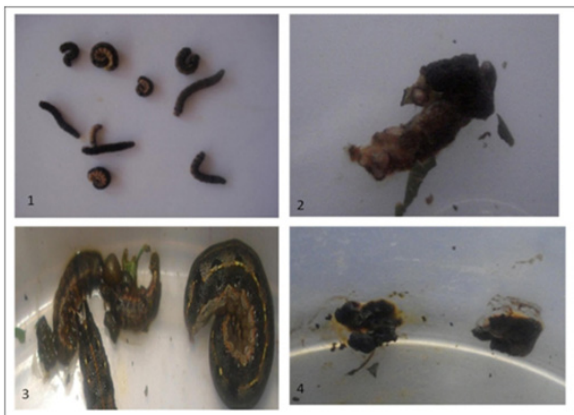
$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{treated leaf}}{\text{Leaf area consumed in control} + \text{treated leaf}} \times 100$$

**Figure 4** Formula for measurement for antifeedant activity.

**Larvicidal and pupicidal activity:** The Larvicidal activity can be studied using leaf dip method.<sup>39</sup> Here the leaf discs of host plant dipped in different concentrations of water extract, synthesized SNP and Leaf extract. The activity is recorded after 24 h of treatment with continuous supply of fresh leaves for healthy growth. The larval mortality rate could be recorded after 96 hours of treatment.<sup>40</sup> The larvicidal (100 %) activities against *H. armigera* with LC<sub>50</sub> value 309.98 mg/mL was recorded by.<sup>38</sup> The percentage of larval mortality was calculated and corrected by Abbott's formula given in Figure 5.<sup>41,42</sup> The mortality of Pest, larvae, pupal can be calculated and effectiveness of particular nanoparticles synthesized using plant extract may be determined. The large number of plant species could be pooled for evaluation of their antipesticidal activity. The in lab experiment for treatment of (1) control, CdS (2), Nano-Ag (3) and (4) Nano-TiO<sub>2</sub> of effected larvae of *S. litura* due to is shown in Figure 6.

$$\text{Corrected Mortality (\%)} = \frac{\% \text{ larval mortality in treatment} - \% \text{ larval mortality in control}}{100 - \% \text{ larval mortality in control}} \times 100$$

**Figure 5** The corrected mortality formula given by Abbott to determine percentage of larval mortality.



**Figure 6** The effected larvae of *S. litura* after the treatment of (1) control, CdS (2), Nano-Ag (3) and (4) Nano-TiO<sub>2</sub> is shown and the picture have been taken from .<sup>42</sup>

**Field test for nano-biopesticides for pest control:** The nano-biopesticide mixed, in particular, quantity of water and sprayed in known quantity in a field of specific selected crop species to study the effect on plants comparing with the standard. The field spraying of nano-biopesticide will need appropriate handling and measurement of nano-biopesticides. The initial quantity of nano pesticide needs to optimized and mixed with water or selected non toxic solvent and sprayed in field. The spraying could be done manually by farmer, automated motors, some time using planes and helicopter based on size of farm. The Figure 7 shows the possible spraying techniques applicable in farms. The overall cost for formulation of silver nitrate, plant collection, extract preparation and lab facilities is very crucial, as it will decide the final cost of nano bio-pesticide formulation. The final comparison of cost for biopesticide and nano bio-pesticide will decide the future of nanobased pesticides. However, the test for their potential is critical aspect for nanotechnologist to established plant protection via nano bio-pesticide. This will conclude the effect of synthesized nano based bio pesticide and it's potential to use as Nano-Bio pesticides as a modern trend.



**Figure 7** Shows the possible spraying techniques applicable in farms A: spraying by human manually, B: through spraying machine and C: using planes in large farm.

## Conclusion

The present articles deals with present pest-control traditional options and discusses the future approach of nano-biopesticides. The problem of pest is not restricted to geographical locations, and the current control measures are very toxic to ecology and human health. The vital challenges faced by pest control are the choice and availability of safe, effective and cheap bio insecticides is need of the day. Furthermore, magnetic nanoparticles could be an alternative for insect repellents with magnetic property helpful in geo-sensing technology. The plant with prominent insecticidal activity could be complexes with Zinc or silver and their efficacy my elevated to control several pests. This will also assist to minimize the most dangerous chemicals used as insecticides today in a field to pests.

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## Conflicts of Interest

The authors declare that they have no conflict of interests; BDL, DPG, SBN idea, designed, plan and wrote the manuscript. The DPG & SBN suggestion and improvement of manuscript. BDL, DPG, SBN discussion, rechecked, revised and finalized manuscript. All authors read and approved the final manuscript.

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