

REVIEW ARTICLE

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# *Bacillus*-based nano-bioformulations for phytopathogens and insect–pest management

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## Abstract

**Background:** Recent concerns linked with the application of chemical pesticides and the increasing necessity of low inputs sustainable agriculture have put the use of microbial biocontrol agents and bio-pesticides to the forefront for their application against plant pathogens and insect–pest management.

**Results:** This review tended to scrutinize the prospects of microbial biocontrol agents and microbes-based nano-formulations against plant diseases and for pest management with emphasis on bacteria-based nanoparticles, especially derived from *Bacillus* species. It also tended to discuss the probable mechanism of action and effect on plant growth along with its prospects in a brief manner.

**Conclusion:** The use of microbial biocontrol agents offers effective, eco-friendly, and long-lasting management of plant diseases. The employment of nanotechnology in the field of biopesticides has emerged as a promising solution. Nano-biopesticides in the form of biologically derived active pesticides or compounds integrated as nanoparticles and integrated into a suitable polymer have application in insect–pest management.

**Keywords:** *Bacillus* sp., Biocontrol, Nanoparticles, Phytopathogens, Insect–pest management

## Background

To meet the escalating need for food demand and security, it is essential to improve the agricultural yields to meet growing population requirements. The control of diseases stands obligatory to safeguard human food sources and agricultural outputs (Syed et al. 2018). Currently, there is a robust thrust towards the development of more sustainable agricultural practices with much lower input. Among these agricultural practices utilizing sustainable alternatives to chemicals for pest and disease management have attained immense potential to alleviate substantial losses of agricultural produce. The alarming adverse effects of conventional

chemicals used for disease and pest management on public health, the environment, and living microorganisms have encouraged researchers to look for a sustainable approach. This has encouraged the exploration of microbial biocontrol agents as a viable alternative for the insect–pest management (Syed et al. 2018). Microbial biocontrol agents offer control over plant diseases and pests by suppressing the plant pathogens' growth using living microorganisms (Köhl et al. 2019). Microbial biocontrol agents defend agricultural products from insect/pest damage through the various mechanism of action including the antagonistic effect on the pathogen, competitive inhibition, and production of antimicrobial metabolites (Köhl et al. 2019). Bacteria are among the most employed biocontrol agent against plant pathogens and pests. Among these, the genus *Bacillus* has been extensively explored because of its entomopathogenic potential (Mampallil et al. 2017). *Bacillus* group of bacteria is an inhabitant of a

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wide number of habitats and is well known with the capability to produce several antimicrobial compounds with varying structures having about 5–8% of genome responsible for the synthesis of secondary metabolites. Biocontrol ability of *Bacillus* strains is demonstrated mainly through an inhibitory action against plant pathogens as well as through the induction of systemic resistance to strive for their population dominance over plant pathogens (Fira et al. 2018).

Nanotechnology offers extensive prospects for the development of materials with improved or inventive functionalities of nano-sized materials and substances due to its much larger surface area. The use of nano-sized materials as the carrier of active ingredients or carriers in the field of pesticides has resulted in improved efficiency in regard to the application as well as dosages required (Devi et al. 2019). The conventional approaches like integrated pest management employed in agricultural practices are insufficient, thereby, creating a demand for alternatives. In this regard, nanotechnology fulfils the demand as an eco-friendly alternative insect-pests management (Bhattacharyya et al. 2016). It is highly employed for the protection of pesticides from hostile environmental factors such as temperature and radiation with considerably improved chemical stability. It enhances the dispensability and wettability of the pesticide formulation and provides a smart delivery system with a controlled release at the targeted site (Kumar et al. 2019a). The recent upsurge of commercial interest in *Bacillus thuringiensis* (*Bt*) has led to enhanced and efficacious formulations. However, its application in the form of nanoparticles (NPs) for plant protection is in juvenile stage and requires further exploration. The development of a nano-formulation based on *Bt* is based on top-down approaches of micronization such as ball and jet milling as well as high-pressure homogenization for the transformation of coarse powder to fine particles with a size range of 2–5  $\mu\text{m}$ . This approach is easy to expand and gives reproducible outcomes, but sometimes may show variation in different batches (Devi et al. 2019). Another important nano-technological approach for insect pest management utilizes the tendency of microbes for the creation of NPs that act antagonistically to pests and insects (Ali et al. 2020).

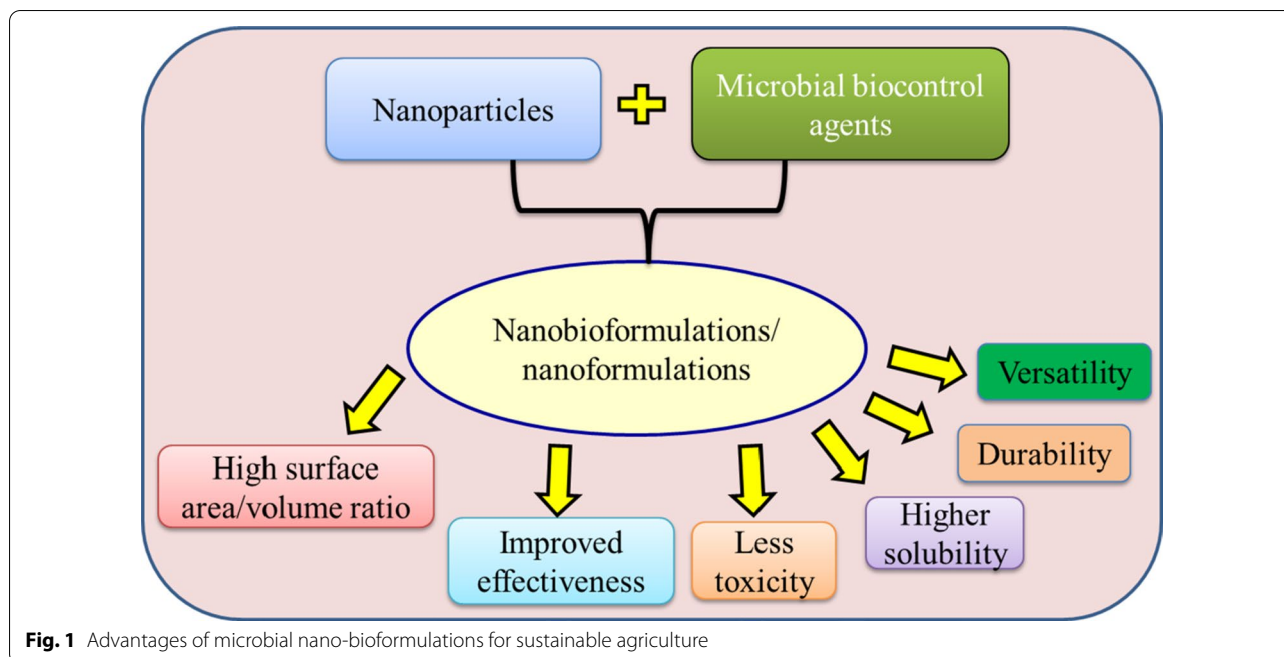
This review tended to provide an updated review on microbial biocontrol agents and nano-biopesticides as an environmentally benign approach against various phytopathogens and insect pest management. It tended to address various bacteria-based NPs or nano-formulations, especially based on *Bacillus* species against phytopathogens and insects along with their mode of action. It also discussed the effect on plant growth and promotion along with the prospects.

## Main text

### Microbial biocontrol

The utilization of chemicals to control diseases in economically essential crops has been practised for a long time and is preferred by the farmers due to its low cost and immediate effect. But their associated adverse effects on human life and other living microorganisms and ability to enter the food chain through the accumulation of toxic residues had encouraged the hunt for more sustainable alternatives. The suppression of plant-pathogen species by living microorganisms is known as “biological control” of plant diseases. Controlling plant pests and pathogens using biological agents seems to be the best choice for developing low-cost, environmentally friendly, and long-term management strategies. Biological control is now widely recognized as an important strategy for mitigating plant disease to ensure sustainable agriculture (Syed et al. 2018). Biocontrol agents include preparations consisting of living microorganisms exerting detrimental effects on the pathogen (Sharma et al. 2017). Isolates of beneficial microorganisms with highly potential effectiveness against pathogens can be grown on culture media. This process of selection and production of antagonists in bulk is referred to as “Augmentative biological regulation” (Köhl et al. 2019). Most of these biocontrol agents include predators and parasitoids that are naturally present which offer exceptional regulation of numerous pests and diseases. Biocontrol agents offer tremendous frontiers as a novel approach, either alone or in the combined application (Patibanda and Ranganathswamy 2018). These microbial biocontrol agents offer several advantages safe to the environment and humans, comparatively less expensive than the conventional agents of chemical origin, wide-spectrum effectiveness against soil-borne pathogens with an increased yield of crop by enhancing beneficial microbes’ growth. The use of biocontrol agent is self-regulating and does not necessitate any complex management, does not exert any toxic effect on plants, long-term disease management and growth-promoting effect (Fig. 1) (Patibanda and Ranganathswamy 2018).

Microbial control agents work in a variety of ways to protect crops from disease disruption. They have the potential to induce enhanced resistance to pathogen infections without having a strong antagonistic relationship with the pathogen apart from their struggle for food and space as an indirect method of action. As for direct mode, hyper-parasitism or antibiosis can be used to deal directly with the pathogen. Hyperparasites attack and destroy fungal pathogens’ mycelia, spores, and resting structures, as well as bacterial pathogens’ cells. Another direct method includes the development of antimicrobial secondary metabolites that inhibit pathogens (Köhl



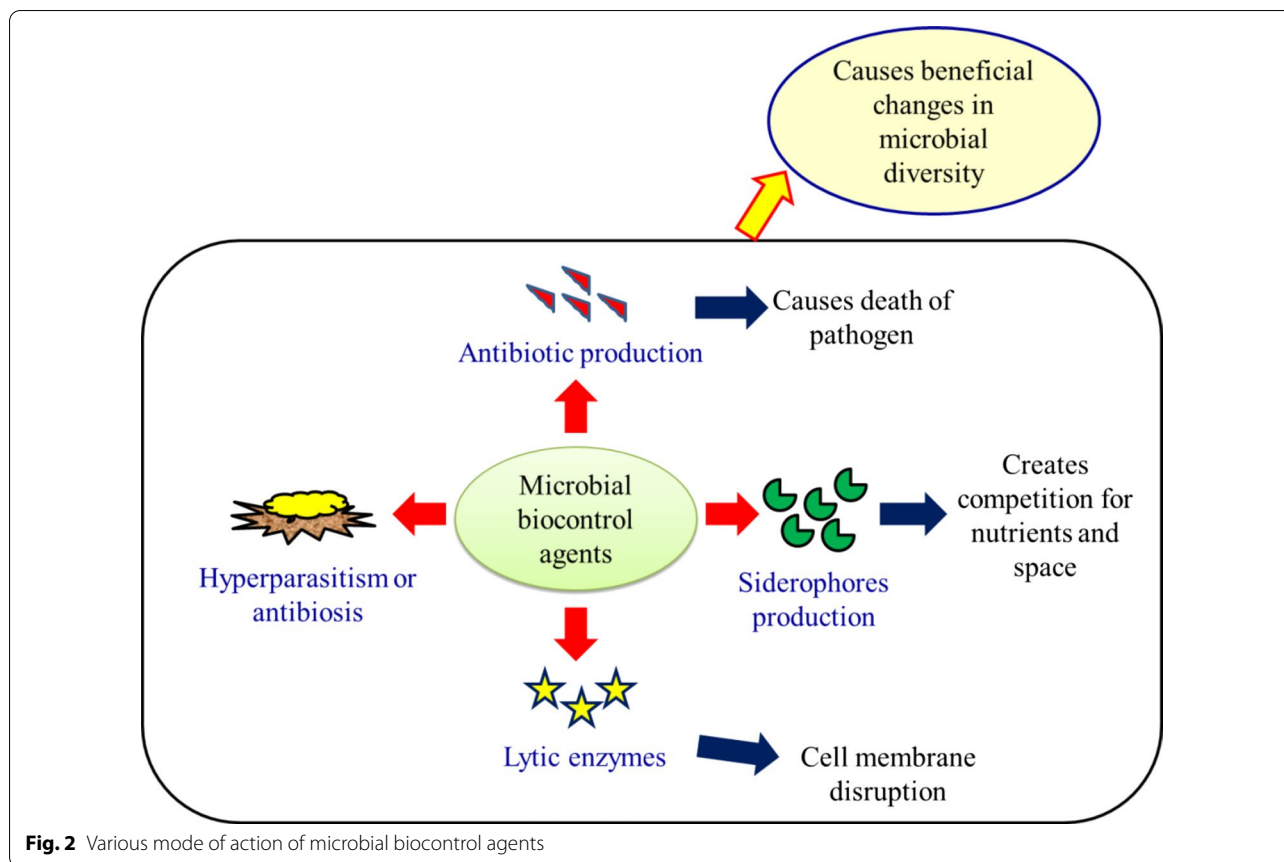
et al. 2019). Antagonists benefit from low levels of secondary metabolites being secreted in situ which help them acquire a competitive lead. In certain cases, biocontrol agents are chosen based on their efficient secondary metabolites production capability in the growth media during mass processing and are used in biological control products alongside or without living antagonist cells. As a result, antagonistic microorganisms can restrict pathogen populations in a variety of ways. The antagonist's mode(s) of action determines more than just how it affects a pathogen population. The microbial biological control agent's characteristics are often influenced based on the mechanism of action employed. The concerns for humans and the environment alongside the development of resistant variety and its reliance on environmental and crop physiological factors may also vary depending on the mechanism of action. Preferences for the specific mechanism of action for a biocontrol agent's intended application would also influence the strategies used to find new antagonists (Fig. 2) (Köhl et al. 2019).

#### Bacteria-based nano-formulation

Bacteria are prokaryotic, unicellular organisms of lengths ranging from a few millimeters to several meters. The Bacillaceae, Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Streptococcaceae families contain the majority of insect pathogenic bacteria. Bacillaceae members, especially *Bacillus* spp., have got a lot of attention as microbial control agents. Bacterial biopesticides are among the most used microbial pesticides working in

a variety of ways. They are often utilized as insecticides, however, can be employed for the mitigation of pathogenic bacteria and fungi in plants. They must encounter the potential pest and ingestion may be a prerequisite to attain effectiveness. Bacteria present in insects inhibit digestion by developing endotoxins. The bacterial biopesticide colonizes the plant and inhibits the disease-causing bacterial or fungal infestation (Kachhawa 2017). The Bacillaceae, Enterobacteriaceae and Pseudomonadaceae families contain the majority of entomopathogenic bacteria. Entomopathogenic activity is also present in households, but to the least degree. Gram-positive, heterotrophic, rod-shaped bacteria that can create endospores make up the Bacillaceae family. *Bacillus thuringiensis*, *B. sphaericus*, *B. popilliae*, *B. pumilus*, *Brevibacillus laterosporus*, and others are members of this genus (Mampallil et al. 2017). *B. thuringiensis* (*Bt*) subspecies and strains are the most commonly used microbial pesticides. *Bt* has been employed for controlling insects and pests in agriculture with its most distinguishing feature that is the formation of crystalline inclusions with insecticidal proteins referred to as "Cry proteins" during sporulation. *Bt* and Cry proteins are considered efficient, reliable, and viable options for insect-pest management (Keswani et al. 2020).

Nanotechnology is a propitious area of interdisciplinary research offering a wide range of applications in the fields of insecticides and agriculture. With growing concerns about the impact of traditional pest management methods on the environment, nanotechnological approaches



**Fig. 2** Various mode of action of microbial biocontrol agents

have offered an environment-friendly alternative for insect–pest management with no or little negative impact on natural harmony. Pathogenic fungi including *Fusarium* spp., *Rhizoctonia solani* and *Phytophthora* spp. infect the whole plant, while *Botrytis cinerea* infects fruit tissues and green parts (Bhattacharyya et al. 2016). Nano-formulations of pesticides must have a wide spectrum of advantages such as enhanced efficiency and longevity, better dispersibility and wettability, biodegradable and free of toxicity with a minimal amount of active ingredients but with convenient pesticidal properties. Researchers have been inspired to produce less toxic and target-specific nano-pesticides without compromising efficacy due to rapid research advances in nano-pesticides. Thus, target-specific nano-pesticides should aid in reducing non-target plant damage and lowering the amount released into the atmosphere (Kumar et al. 2019b).

Bacterial tendency to reduce metal ions through active absorption has promoted their utilization for the creation of antagonistic nanoparticle synthesis for insect–pest management. Several bacterial species (*Bacillus* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Shewanella* sp.) have been utilized for the synthesis of NPs using various inorganic metals (e.g. Ag, Al, Au, MnO, ZnO, TiO<sub>2</sub>) over

the last two decades. The use of these bacteria-mediated NPs has improved agricultural production by reducing losses during crop processing, transportation and storage (Kumari et al. 2019). Few examples of bacteria-based nano-formulations and their applications against phytopathogens and insect–pest management are listed in Table 1.

#### ***Bacillus* sp. against phytopathogens and insect pest**

The microorganisms from the *Bacillus* group secrete various kinds of compounds with antagonistic properties which exist in various habitats. An effective compound of the antagonistic organisms can be selected based on the principle step of the biological control (Ashwini and Srividya 2014). The *Bacillus* species produce approximately 8% of the total secondary metabolites with antagonistic properties. The majority of the activities including siderophores, lipopeptides, bacteriocins, and polyketide compounds from the *Bacillus* species are synthesized non-ribosomically (Fira et al. 2018). The *Bacillus* species such as *B. stearothermophilus*, *B. laterosporus*, *B. circulans*, *B. licheniformis*, *B. amyloliquefaciens*, *B. pabuli*, *B. magaterium*, *B. thuringiensis*, and *B. subtilis* are known to secrete chitinase. Chitinase from the *B. subtilis* had

**Table 1** Bacteria-based nano-formulations (nanoparticles/lipopeptides) and their applications against phytopathogens and insect-pest management

Bacterial species	Nano-formulation nanoparticles	Target pathogen	Application	References
<i>Bacillus amyloliquefaciens</i> and <i>Bacillus subtilis</i>	Ag NPs	<i>Culex pipiens pallens</i>	Quick effect on vector mosquito and microbial pathogen	Fouad et al. (2017)
<i>B. cereus</i> SZT1	AgNPs	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Effective against leaf blight in rice caused by bacteria	Ahmed et al. (2020)
<i>B. cereus</i> RNT6	ZnO NPs	<i>Burkholderia glumae</i> and <i>B. gladioli</i>	ZnONPs could be used as nano-pesticides against rice panicle blight	Ahmed et al. (2021)
<i>B. thuringiensis</i>	AgNPs	<i>Trichoplusia ni</i> and <i>Agrotis ipsilon</i>	Btk-synthesised AgNP showed high virulence toward larvae of <i>Trichoplusia ni</i> than to <i>Agrotis ipsilon</i>	Sayed et al. (2017)
<i>B. thuringiensis</i> coated	ZnO NPs	<i>Callosobruchus maculatus</i>	Bt-ZnO based NPs effective against <i>Callosobruchus maculatus</i>	Malaikozhundan et al. (2017)
<i>B. thuringiensis</i>	Au NPs	<i>Aedes aegypti</i> and <i>Anopheles subpictus</i>	AuNPs and Bt toxin interaction able to protect Bt from sunlight as well as relocate Bt toxin to the target site	Patil et al. (2018)
<i>Paenibacillus polymyxa</i> strain Sx3	ZnO, MnO <sub>2</sub> , and MgO	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> (Xoo)	Combat the antibiotic resistant of Xoo	Ogunyemi et al. (2020)
<b>Lipopeptides</b>				
<i>B. amyloliquefaciens</i>	Bacillomycin D	<i>F. oxysporum</i> f. sp. <i>cucumerinum</i>	<i>Fusarium</i> wilt control of cucumber	Koumoutsis et al. (2004), Xu et al. (2013), Li et al. (2014)
<i>B. circulans</i>	Iturin A	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	Management of <i>Fusarium</i> crown and root rot of tomato	Hsieh et al. (2008)
<i>B. licheniformis</i>	Surfactin	<i>Magnaporthe grisea</i>	Effective against rice seedling blight	Tendulkar et al. (2007)
<i>B. mojavensis</i>	Mojavensin A	<i>F. oxysporum</i> f.sp. <i>cucumerinum</i> , <i>F. verticillioides</i> and <i>Valsa mali</i>	Effective in <i>Fusarium</i> wilt control of cucumber and rotting and wilting of maize	Ma et al. (2012)
<i>B. pumilus</i>	Pumilacidin	<i>Pythium aphanidermatum</i> , <i>Rhizoctonia solani</i> and <i>Sclerotium rolfsii</i>	Effective against rotting of soybean, root and stem, and blights of fruits	de Melo et al. (2009)
<i>B. subtilis</i>	Iturin/Fengycin	<i>Podospaera fusca</i>	Protection against powdery mildew in cucurbits	Romero et al. (2007), Meena and Kanwar (2015)
	Iturin A/Fengycin	<i>Penicillium digitatum</i>	Green mold disease management in mandarin fruit	Waewthongrak et al. (2015)
<i>B. thuringiensis</i>	Bacillomycin Ls/Fengycin	<i>Fusarium oxysporum</i>	Control of <i>Fusarium</i> wilt	Luo et al. (2015)
	Kurstakin	<i>Colletotrichum gloeosporioides</i>	Anthraxnose disease control in fruits	Kim et al. (2004), Mnif and Ghribi (2015)
<i>B. vallismortis</i>	Bacillomycin D	<i>Alternaria alternata</i> , <i>Cryphonectria parasitica</i> , <i>F. graminearum</i> , <i>Phytophthora capsica</i> , and <i>Rhizoctonia solani</i>	Mitigation of wheat and vegetable diseases	Zhao et al. (2010)

premier chitinolytic actions that can degrade the chitin zones in colloidal chitin. The chitinase enzyme shows antifungal activities against *Aspergillus flavus*, *A. niger*, and *Penicillium chrysogenum*. The *Bacillus* strain BG11 secretes chitinase with some specific properties including higher production rate at stationary phase, competent

monomeric degradation of chitin, less inhibition of the end product, resistance against salt and proteases, and compatibility with many pesticides as compared with the other *Bacillus* species (Karunya et al. 2011). The chitinase enzymes can break the hard chitin cell wall of the conidia, hyphae, sclerotia, and chlamydospores and also

have inhibitory actions against many plant pathogens. Other species like *B. cereus*, *B. mycoides*, *B. pumilus*, *B. pasteurii*, *B. sphaericus*, and *B. mojavensis* including the above species are efficiently producing the antibiotic molecules with the inhibitory actions for plant pathogens by antibiosis mechanism (Cawoy et al. 2011). The strains of the *B. subtilis* named PRBS1 and AP3 had been reported for in vitro inhibition of the pathogenic fungi from the soybean seeds including species of *Phomopsis*, *Colletotrichum truncatum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Sclerotinia sclerotiorum*. Hence, *B. subtilis* strains are potentially used to treat pathogens of the seeds or to promote plant growth (Widnyana and Javan-dira 2016).

NKG1-a strain of *Bacillus methylotrophicus* has been used for its potential to treat fungal infections and also used as a fertilizing agent. This strain was extracted from the root area of *Pinus koraiensis* (Ge et al. 2016). The strain of *B. subtilis* BC2 demonstrated antagonistic activity against pathogenic fungi through the lytic process mediated by enzymes. The other studies showed that the BC2 strains acted as a biological controlling agent against *Colletotrichum gloeosporioides* (Ashwini and Srividya 2014). Under greenhouse, the native strains of the *Bacillus* species B19 and P12 can reduce the white mold in French beans caused by *Sclerotinia sclerotiorum* (Sabaté et al. 2018). The other species of the *Bacillus* species strains including *B. amyloliquefaciens* 17A-B3 and *B. subtilis* 30B-B6 have worked against *Phytophthora infestans*. Throughout the crop season, the late blight disease was notably cured by the *B. subtilis* strain 30B-B6 (Caulier et al. 2018). The lipopeptides from the *B. subtilis* can reduce the growth of pathogenic species of microbes including *Aspergillus*, *Rhizopus*, *Botrytis*, and *Penicillium* (de Andrade et al. 2019). *B. thuringiensis* is the widely studied species of the *Bacillus* group against pesticides. The insects from various orders such as Coleoptera, Diptera and Lepidoptera can be controlled biologically by using the accumulated protein crystals synthesized by *B. thuringiensis* (Valtierra-de-Luis et al. 2020). Chitinase enzymes secreted from the *Bt* can hydrolyze the chitin. Chitinase enzymes can enhance the insecticidal properties of Cry proteins. *Bt* chitinases have the potential to control nematodes, fungi, and entomopathogens and are also used to produce chitin-derived oligosaccharides that possess antibacterial properties (Martínez-Zavala et al. 2020).

#### ***Bacillus* sp.-based nanoparticle synthesis/ nano-formulation**

Recently in the area of biology and medicines, noble nano-formulations are preferred due to their unique morphology (shape and size) that further depends on

biological, chemical, and physical factors. The synthesis of NPs has been done by using different processes based on biological, chemical, and physical approaches (Gopinath and Velusamy 2013). The most preferred methods of nanoparticle synthesis should offer superior properties of the encapsulated material and be cost-effective with no toxic hazards (Ghiuță et al. 2018). In the clinical and food sector, the chemical approach-based NPs were evaded because their toxicity came from the chemicals, which were used during formulation. The nano-formulations based on the biogenic approaches (cheap and eco-friendly) took priority due to the elimination of the chemical toxicity (Wei et al. 2012). Based on the place of nano-formulation, there are two main categories of the biosynthetic approaches—*intra* and *extracellular* synthesis. The *extracellular* synthesis of NPs is an emerging approach to get insights on the mode of synthesis along with down- and upstream processing. The living microorganism-based biopesticides had the potential to manage pest control (Senthil-Nathan 2015).

One of the *Bacillus* species strain GP23 extracted from the marine soil was used to prepare silver nanoparticles (AgNPs) by the *extracellular* approach which suppressed the *Fusarium oxysporum* was responsible for the wilt disease of legumes, banana, tobacco, cucurbits, sweet potatoes, and tomato (Gopinath and Velusamy 2013). The *extracellularly* synthesized AgNPs by using the SBT8 strain of *Bacillus* have antimicrobial properties and acted as a biocatalyst (Yurtluk et al. 2018). The NPs synthesized taking silver nitrate as precursor using *B. subtilis* and *B. amyloliquefaciens* showed antibacterial activity against both gram-positive and negative bacteria as well as antifungal actions against *Candida albicans* (Ghiuță et al. 2018). The nanoscale *Bt* chitinases exhibited excellent capability for the deliverance of the nematicidal preparations for managing *Caenorhabditis elegans*. Hence, the nanoscale *B. thuringiensis* chitinases can act as a biopesticidal delivery system to extend farming applications (Qin et al. 2020). By applying the nano-technological approach, the size of the entomopathogenic bacterium (*Bt*) can be reduced to formulate *Bt* nanoparticle, which was used as a biocontrol agent (Swamy and Asokan 2013). Instead of NPs, the nano-composites of *Bt* with *Bt* bioactives in various nano-formulations such as nanocapsules, nano-suspension, and nano-emulsion had been utilized as nano-pesticides (Damalas and Koutroubas 2018).

The Cry protein extracted from the *Bt* is the major component that belongs from the large families of protein possessing toxic activities with different specifications. Most of this show toxicity against dipterans, coleopterans, and lepidopterans, few of these proteins also work against nematodes and hymenopterans. The

loading of Cry proteins to the nanomaterial for the production of the nano-composites could be easy and the bioactivity of the Cry protein was maintained by the nano-materials which also enhanced the stability (Devi et al. 2019). The nano-crystallization of the cyclic lipopeptides extracted from the *B. subtilis* enhances the stability at storage conditions by oxidation prevention and antifungal activity by controlled deliverance of the cyclic lipopeptides. These nano-crystals (solid lipid NPs) of the cyclic lipopeptides also prevent the growth of the *Aspergillus carbonarius* and *A. niger* spores and hyphae (Lin et al. 2020). The zero-valent AgNPs had been synthesized using *Bacillus* species strain AW1-2 to control *Colletotrichum falcatum* which is responsible for the red rot disease of sugarcane (Ajaz et al. 2021).

#### **Mechanisms of action of nano-particles/nano-formulation against phytopathogens**

Many variations were found in the mode of biocidal action of nano-formulations based on the employed microbes including parasitism, antibiosis, competence in rhizosphere-like stimulation, and alteration in plant growth and metabolism, respectively (Shrivastava et al. 2014). The nano-formulations are known to release bio-agents from the NPs in a targeted manner. Many nano-pesticides have been produced which were used to prevent the most effective plant pathogen including *Phylosticta*, *Phytophthora*, *Aspergillus*, *Fusarium*, and *Phoma* species (Bhattacharyya et al. 2016). The nano-formulations can act as a plant growth promoter or disease suppressor by activating the various defense mechanisms (Malerba and Cerana 2018). The NPs acted against phytopathogens through disruption of protein, formation and antioxidant deprivation of the reactive oxygen species (ROS), membrane impermeability, and inhibition of the gene transporter. All of the above pathways were dependent on each other and acted in combination with different phytopathogens (Alkhattaf 2021).

The negative charge of the NPs and electrostatic interactions of microbial membranes can adhere to each other. The NPs and membrane depolarization have disrupted the respiratory and permeability actions of the membrane that can affect the physical properties and had disrupted the cell components of the phytopathogen that ultimately leads to cell death. The disruption of the cell membrane results in the outflow of cellular constituents such as proteins, DNA, RNA, enzymes, and metabolites. In the intra and extracellular regions of the cell wall (phytopathogens), many irregular gaps can be created by the NPs for their dispersion (Alghuthaymi et al. 2015). The damaging of the cell membrane and perforations of the cell surface caused by NPs had been observed through transmission electron microscopy. The Transmission

Electron Microscopy (TEM) observations showed the severe damage of the cell membrane with inflammation and discharge of the cytoplasmic and nuclear components resulting death of the pathogens (Ibrahim et al. 2020). The plant fungi like *Trichosporon asahii* and *Alternaria alternata* also found similar effects of AgNPs. In these species, NP toxicity can be the consequence of the formation of ROS (Xia et al. 2016). The ROS formed during the mode of action of NPs can disrupt the cell membrane and other cellular components like fat, proteins, RNA, and DNA molecules. In the cells of the *Azotobacter vinelandii*, the cellular damage and AgNPs had detected by the TEM and also found the hydroxyl radicals developed by the AgNPs by electron spin resonance (Alkhattaf 2021).

#### **Mechanisms of action of nano-particles/nano-formulation against insect pest**

In many nano-formulations, which had been used against the *Tribolium castaneum*, muscular destruction, changes in the pigmentation, epidermal thickness, and necrosis were found in the cuticle and also found in the cellular destructions among the endo and exocuticle (Hashem et al. 2018). In addition to the injury in the cuticle, the AgNPs made up of seed extract of *Pedalium murex* caused loss of the hairs from the head, abdomen, and antenna of the larvae of *Aedes aegypti* (Ishwarya et al. 2017). Additionally, the AgNPs can also cause cuticle depigmentation. Moreover, the AgNPs were also reported to create stress which can increase the level of cytokines, ROS, alteration in membrane potential, and pro-inflammatory mediators (Mao et al. 2018). The impairment of the crawl and climb abilities in later and adult stages has been found if the ingestion of the AgNPs occurred in the early stages of the larva. The metabolic alterations in fat, carbohydrates, and proteins with a reduced level of fat droplets, and an enhanced level of ROS had been found in the larval tissues if the oral dosage of the AgNPs increased (Raj et al. 2017).

If the flies are treated with a nonlethal concentration of AgNPs, the depigmentation in the cuticle was found which resembled the copper starvation conditions. In this condition, the enzymatic activities of the two copper-dependent enzymes namely Cu-Zn superoxide dismutase and tyrosinase were decreased. These enzymes augmented pigment production and antioxidant actions (Armstrong et al. 2013). The formation of ROS occurred due to the toxic components and further elevates the immune system and superoxide dismutase which helped the insect to manage the extracellular ROS (Gretschner et al. 2016). The expression of the protein calxectin accountable for the excitement of the membrane and calcium-binding had been lowered by applying AgNPs

(Meng et al. 2017). Other nano-formulations enhanced the activity of the acetylcholine esterase and glutathione S-transferases (Milivojevic et al. 2015). The inhibition of the protease actions had been found because of the AgNPs (Kantrao et al. 2017). The protease inactivation was occurred due to the electrostatic and hydrophobic interaction which further endorsed the protease adsorption on the NP surface that can convert the biological actions of the enzyme, for example, by impairing the binding with the substrate (Shahzad and Manzoor 2021).

### Effects on plant growth and promotion

Nanoparticles hold specific physical and chemical characteristics that can alter the biochemical and physiological processes of the plant during growth and promotion. The reduced weight of the seeds and increased number of the seeds was found by applying the NPs on the shoot and root growth with the involvement of mycorrhizal fungi and growth-promoting microorganisms. The treatment of the seeds with NPs modulated the phytohormones and participation of the other cellular components during germination. The growth induction was found in the *Dracocephalum moldavica* plants by applying NPs (Gohari et al. 2020). Among the other species of the *Bacillus*, many *B. subtilis* strains in the form of silver, gold, and aluminium-coated NPs can significantly increase plant growth by inhibiting the phytopathogens and insect pests, hence used as potential nano-bio-fertilizers (Gouda et al. 2018). The biocontrol effect included the induction ability of the natural host plant defense system for the various phytopathogens (Lastochkina et al. 2018). Further, the formulation and storage of the *B. subtilis* strains were easy, because they can produce endospores with resistant properties against physical and chemical factors including pH, temperature, desiccation, UV radiation, and consequently, could activate and maintain the plant host defense system under adverse circumstances. The V26 strain of the *B. subtilis* exhibited higher activity against phytopathogens with potential components for biocontrol and also played a vital role in plant growth and promotion (Shahzad and Manzoor 2021).

### Conclusion

Bio-pesticides are gaining popularity around the world as a better way for insect-pest management with minimum risks to humans and the environment. Biopesticides based on nanotechnology provide environmentally friendly and effective pest control options that are not harmful to the environment. Biopesticides, especially nano-biopesticides, have the potential to metamorphose the global agricultural field in terms of food and feed protection. Nano-biopesticides excel

because of their small size, large surface area, durability, increased effectiveness, high solubility, versatility, and low toxicity. Nano-biopesticides have the potential to reduce the toxicity of chemical pesticides, while also providing target-specific crop pest control. They may be useful in the creation of intelligent nano-systems for the reduction of agricultural problems including environmental effects, food productivity, safety and security. These nano-systems have demonstrated a high capacity for controlled-release behaviour, thereby assuring their effectiveness in long-term use with the possibility of addressing the issues of eutrophication and pesticide residues' accumulation.

But the most significant stumbling block to nano-technological agricultural progress lies with its ethical acceptance. Farmers in India are poorly trained and do not know about nano-technological agricultural facilities. Toxicity and/or NPs accumulation in biological processes such as the food chain is another great concern. In addition, the NP-based agricultural research is of interest due to the delayed exposure of NPs in the natural system. Scientists are, therefore, trying to figure out how to respond to the acceptance of NPs and their non-absorption in a cell or system that ultimately leads to their further amplification. Hence the future research needs to target better understanding of the characterization, formulation, morphology as well as application of nano-biopesticides to apprehend their final fate in animals, humans, and plants. As a result, various aspects of nano-biopesticides, including their present status, constraints, prospects, and regulatory network, must be checked regularly to ensure their successful use for the benefit of humanity. In addition, studies investigating at molecular levels using various animal models should be focused on to fully illustrate the mode of actions involved in insect-pest management. Further, the long-term effects of the use of NPs on plants and animals should be evaluated along with their stability, quantity, and crop-specific application dosages to ensure agricultural safety and sustainability.

### Abbreviations

NPs: Nanoparticles; AgNPs: Silver nanoparticles; AuNPs: Gold nanoparticles; MgO: Magnesium nanoparticles; MnO<sub>2</sub>: Manganese dioxide; ZnO NPs: Zinc oxide nanoparticles; Al: Aluminium; MnO: Manganese oxide; ZnO: Zinc oxide; TiO<sub>2</sub>: Titanium dioxide; TEM: Transmission electron microscopy; ROS: Reactive oxygen species.

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### Authors' contributions

PK conceived and designed the manuscript; DKM, SP, MK and PK wrote the manuscript; PK and AM critically reviewed the manuscript and did the required editing. All authors have read and approved the manuscript.



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Authors declare that they have no competing interests.

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