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Microorganisms as a biopesticides

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Abstract
Microbial pathogen consists of disease causing organism, which are disseminated in the pest population in large quantity in a manner similar to application of chemical pesticides. These pathogens are exploited for biological control of insect pests through introductory or inundative applications. Insects like other organism are susceptible to a variety of diseases caused by different groups of microorganisms including virus, bacteria, fungi, protozoa and nematodes. Microbial pathogens of insects are intensively investigated to develop environmental friendly pest management strategies in agriculture. In the present day plant protection scenario, development of resistance to chemicals and residue in higher trophic level are major hurdle in insect pest management. In the recent years biopesticides are replacing the chemicals pesticides to overcome the harmful effect of the chemicals on non-target organism. This paper reviews the insecticidal properties of microbes and their potential utility in pest management.

Keywords: Microorganisms, biopesticides, microbial pathogen, biological, pest population

1. Introduction

Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects which have serious effect on feed production as global crop yield is reduced by 20 to 40% annually due to plant pest and diseases [47]. With the advent of chemical pesticides, this crisis was resolved to a great extent. But the over dependence on chemical pesticides and eventual uninhibited use of them has necessitated for alternatives mainly for environmental concerns. Though biopesticides cover about 1% of the total plant protection products globally, their number and the growth rate have been showing an increasing trend in the past two decades [48] about 175 biopesticides active ingredients and 700 products have been registered worldwide. Among various bio products *Bacillus thuringiensis* (Bt), *Trichoderma viride*, *Metarhizium* spp., *Beauveria bassiana* and Nuclear polyhedrosis virus are popularly used in plant protection [49]. Among them most successful insect pathogen used for insect control is the bacterium, *Bacillus thuringiensis (Bt),* which presently occupies about 2 per cent of the total insecticidal market [50]. The most widely used bacterial pathogens include subspecies or strains of *Bacillus thuringiensis.* Each one of the strains produces different mix of toxins and specifically kills one or a few related species of insects (*Bt* subspecies *kurstaki* and *aizawai* for lepidopteran larvae and *Bt* subspecies *tenebrionis* for coleopteran larvae). Some of these strains are specific to mosquitoes (*Bt* subspecies *israelensis*). Among the insect viruses baculoviruses (Nuclear polyhedrosis virus, NPV and Granulosis virus, GV) are the most promising for insect control particularly of Lepidoptera and Diptera because of their specificity. NPVs have been successfully used for management of devastating pests like *Heliothis* spp. and *Spodoptera* spp. on cotton, fruit and vegetable crops in several countries [51]. Entomopathogenic fungi like *Beauveria* spp., *Metarhizium* spp., *Lecanicillium* spp. and *Isaria* spp. have been developed as successful mycoinsecticides for various groups of insect pests [52]. Several hundreds of commercial products of fungi, bacteria and viruses are available worldwide for the biological control of insect pests in agriculture and forestry. The growth rate of the bio-pesticide industry has been forecasted in the next 10 years at 10-15 per cent per annum in contrast to 2-3 per cent for chemical pesticides [53]. The main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organism, they do not cause ill effects on environment and human health and can be used against pests which develop resistance to the conventional insecticides, and they fit as ideal components in integrated pest management (IPM). In this paper, a review is made on the prospects of utilization of insect pathogens in pest management worldwide and in India.

2. Biopesticide

As defined by the United States Environmental Protection Agency (EPA), biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals. In commercial terms, biopesticides include microorganisms that control pests (microbial pesticides), naturally-occurring substances that control pests (biochemical pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants). Biopesticides are employed in agricultural use for the purposes of insect control, disease control, weed control, nematode control and plant physiology and productivity. The EPA separates biopesticides into three major classes based on the type of active ingredient used, namely, biochemical, plant-incorporated protectants and microbial pesticides (USEPA 2008). Within each of these, there are various types of products, each with its own mode of action.

3. Microbial pesticides

Microbial pesticides are also known as Biological Control Agents. In this category, the active ingredient is a microorganism that either occurs naturally or is genetically engineered. The pesticidal action may be from the organism itself or from a substance it produces. They offer the advantages of higher selectivity and less or no toxicity in comparison to conventional chemical pesticides [2]. The most commonly used microbial biopesticides are living organisms, which are pathogenic for the pest of interest. These include biofungicides (*Trichoderma*, *Pseudomonas*, *Bacillus*), bioherbicides (*Phytophthora*), and bioinsecticides (*Bt*) [3]. Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or alga, rickettsia, Mycoplasma and nematodes) as the active ingredient. They suppress pests either by producing toxic metabolites specific to the pest, causing disease, preventing establishment of other microorganisms through competition, or various other modes of action [46]. They suppress pest by producing a toxin specific to the pest causing a disease. Preventing establishment of other microorganisms through competition or other modes of action. Some of the pathogens may be quite common and are frequently the cause of epizootics in natural insect populations while others are rarely observed [4].

3.1 Bacteria

Bacteria are prokaryotic, unicellular organism varying from less than 1µm to several length. Most of the insect pathogenic bacteria occur in the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae and Micrococcaceae. Member of Bacillaceae, particularly *Bacillus* spp., have received maximum attention as microbial control agents [5]. Bacterial biopesticides are the most common form of microbial pesticides that function in multiple ways. Generally, they are used as insecticides, although they can be used to control the growth of plant pathogenic bacteria and fungi. As an insecticide, they are generally specific to individual species of moths and butterflies or species of beetles, flies, and mosquitoes. To be effective, they must come into contact with the target pest and may be required to be ingested. In insects, bacteria disrupt the digestive system by producing endotoxins that are often specific to the particular insect pest. When used to control pathogenic bacteria or fungus, the bacterial biopesticide colonizes on the plant and crowds out the pathogenic species [6]. The most widely used microbial pesticides are subspecies and strains of *B. thuringiensis* (Bt), accounting for approximately 90% of the biopesticide market in the USA [7]. Bt has been widely used to control insect pests important in agriculture, forestry, and medicine [8]. Its principal characteristic is the synthesis, during sporulation, of crystalline inclusions containing proteins known as δ endotoxins or Cry proteins, which have insecticidal properties. Due to their high specificity and safety in the environment, *B. thuringiensis* and Cry proteins are efficient, safe, and sustainable alternatives to chemical pesticides for the control of insect pests [9].

Bt variety	Target pest
B. popilliae	Japanese beetle grubs
Bacillus sphaericus	Mosquito larvae
B . thuringiensis subsp. aizawai	Moth larvae
B. thuringiensis subsp. israelensis	Mosquito and blackflies
B. thuringiensis subsp. kurstaki	Lepidopteran larvae
B. thuringiensis subsp. tenebrionis	Colorado potato beetle
B. thuringiensis subsp. galleriae	Lepidopteran larvae
Bacillus moritai	Diptera

Source: Kunimi (2007) and Kabaluk *et al.* (2010) [10, 11]

3.2 Viruses

A virus is a set of one or more nucleic acid template molecule, normally encased in a protective coat of protein or lipoprotein that is able to organize its own replication only within suitable host cells. Viruses have been isolated from more than 1000 species of insects from at least 13 different insects order [12]. Entomogenous viruses fall into two categories, viz. inclusion viruses (IV) producing inclusion bodies in the host cells and non-inclusion viruses (NIV) which do not produce inclusion bodies. The IV are further sub divided into polyhedron viruses (PV) or polyhedroses, which produce polyhedral bodies and granulosis virus which produce granular bodies. Polyhedroses could inhabit the nucleus and are called nuclear polyhedrosis viruses (NPV) or the cytoplasm which are called cytoplasmic polyhedrosis virus (CPV) [18]. Among the insect viruses found in nature, those belonging to the baculovirus family (Baculoviridae) were considered for the development of most commercial viral biopesticides [13-15]. Members of this family are regarded as safe for vertebrates and, to date, no cases of pathogenicity of a baculovirus to a vertebrate have been reported [16,17]. Baculoviruses are insect-specific, enveloped viruses with circular, supercoiled double-stranded DNA genomes in the range of *ca.* 80–180 kbp [18]. More than 600 baculoviruses have been isolated from Lepidoptera (butterflies and moths), Hymenoptera (sawflies), and Diptera (mosquitoes) [19]. The name "baculovirus" is derived from the rod-shaped, nucleocapsids (Latin "*baculum*": stick) which are 230–385 nm in length and 40–60 nm in diameter [18]. Baculoviruses are infectious *per os* (by mouth) and exhibit efficient horizontal transmission. When OBs are consumed by insect, the alkaline environment of the midgut triggers the dissolution of polyhedra (OBs) and the release of virions into the midgut lumen [20].The virions enter the midgut cell nucleus, at which point the virus replicates within the nuclei of susceptible tissue cells and tissue susceptibility varies greatly between virus with some NPVs being capable of infecting almost all tissue types and most GVs being tissue specific replications (fat bodies cell only). The budded virus initiates infection to other tissues in the hemolymph, i.e. fat bodies, nerve cells, haemocytes, etc. The cell infected in the second round of virus replicate in the insect larva also produce budded virus but in addition occlude virus particles within polyhedral in the nucleus. The accumulation of polyhedral within the insect proceeds until the host consists almost entirely of a bag of virus. In the terminal stage of infection, the insect liquefies and thus releases polyhedral, which can infect other insects upon ingestion. A single caterpillar at its death may contain over 10^9 occlusion bodies from an initial dose of 1000. The infected larvae exhibit negative geotropism before succumbing to the virus infection, thereby facilitating widespread dissemination. The speed with which death occurs is determined in part by the environmental conditions. Under optimal conditions, targets pests may be killed in 3-7 days, but death may be caused in 3-4 weeks, when conditions are not ideal [21].

Nature of virus	**Host**
Nuclear polyhedrosis virus	*Helicoverpa armigera, Spodoptera litura, Amsacta albistriga, Spilosoma obliqua, Pericallia ricini, Pseudaletia separata Spodoptera mauritia, Corcyra cephalonica, Plusia chalcites, Antheraea mylitta, Dasychira mendosa, Plusia peponis*
Granulosis virus	*Cnaphalocrocis medinalis*, *Pericallia ricini, Achaea janata, Phthorimaea operculella* and *Chilo infuscatellus*
Cytoplasmic polyhedrosis virus	*Helicoverpa armigera*
Pox virus	*Amsacta moorei*

(**Source:** Ramakrishnan and Kumar, 1976) [22]

3.3 Fungi

Entomopathogenic fungi are considered to play vital role as biological control agent of insect populations [23]. A very diverse array of fungal species is found from different classes that infect insects. These insect pathogenic species are found in a wide range of adaptations and infecting capacities including obligate and facultative pathogens [24]. Spreading of fungal diseases is common in many insect species while some species may not be affected. In 1980s, the first insect pathogenic studies were carried out and their focus was to find the methods of disease management of the silkworm [25]. Bassi in 1835, first time formulated the germ theory by the use of white muscardine fungus on the silkworm that was then named in his honor as *Beauveria bassiana*. Gilbert and Gill [26] described that this silkworm disease gave the idea of using insect infecting fungi for the control of insect pest management. Many commercial products are available globally [27] that are formulated by utilization of less than ten species of fungi [28]. The divisions of fungi are Ascomycota, Zygomycota and Deuteromycota [29], and the divisions Oomycota and Chytridiomycota were also included in the previous classification of fungi. At the recent times, about 90 genera and almost above 700 species are considered as insect infecting fungi that represent about all the major classes of fungi [30, 31]. A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi [32]. The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea [33]. Entomopathogenic fungi have a great potential as control agents, as they constitute a group with over 750 species and when dispersed in the environment, provoke fungal infections in insect populations. These fungi begin their infective process when spores are retained on the integument surface, where the formation of the germinative tube initiates, the fungi starting to excrete enzymes such as proteases, chitinases, quitobiases, Upases and lipoxygenases. These enzymes degrade the insect's cuticle and help in the process of penetration by mechanical pressure that is initiated by the appressorium, a specialized structure formed in the germinative tube. Once inside the insect, the fungi develop as hyphal bodies that disseminate through the haemocoel and invade diverse muscle tissues, fatty bodies, Malpighian tubes, mitochondria and haemocytes, leading to death of the insect 3 to 14 days after infection. Once the insect dies and many of the nutrients are exhausted, fungi start micelles growth and invade all the organs of the host. Finally, hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions [24].

Fungi	Target
Beauveria bassiana (muscardine fungus)	Colorado potato beetle, Corn root worm, Citrus root weevil, Cotton bollworms, Coffee berry borer, codling moth, Japanese beetle, Pod borer, Mango mealy bug, Boll weevil, Cotton leaf hopper, Chinch bug, Yellow stem borer, Rice leaf folder, Brown plant hopper, etc.
Metarhizium anisopliae	Spittle bug, Sugarcane hopper, Rhinoceros beetle, Termite, Locust, Grasshoppers, etc.
Hirsutella thompsonii	Phytophagous mites
Verticillium lecanii	Aphid, Whiteflies and Scales
Nomuraea rileyi	*Helicoverpa armigera, Spodoptera litura, Trichoplusiani* and *Achaea janata*
Aschersonia aleyroides	Whitefly
Pandora delphacis	Brown plant hopper and green leaf hopper of rice
Phytophthora palmivora	Milk weed vine (weed)
Alternaria cassiae	Sickle pod (weed)
Colletotrichum gloeosporioides	Northern joint vetch (weed)
Fusarium lateritium	Velvet leaf (weed)

(**Source:** Pawar and Singh 1993 and Zimmermann, 1993) [34, 35]

3.4 Nematodes

Entomopathogenic nematodes are soft bodied, nonsegmented roundworms that are obligate or sometimes facultative parasites of insects. Entomopathogenic nematodes occur naturally in soil environments and locate their host in response to carbon dioxide, vibration, and other chemical cues [36]. Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs [37]. Entomopathogenic nematodes fit nicely into integrated pest management, or IPM, programs because they are considered nontoxic to humans, relatively specific to their target pests, and can be applied with standard pesticide equipment [38]. Entomopathogenic nematodes have been exempted from the US Environmental Protection Agency (EPA) pesticide registration. There is no need for personal protective equipment and re-entry restrictions. Insect resistance problems are unlikely. The infective juvenile stage (IJ) is the only free living stage of entomopathogenic nematodes. The juvenile stage penetrates the host insect via spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle, and then enters into the hemocoel [39]. Both Heterorhabditis and Steinernema are mutualistically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively [40]. The juvenile stage release cells of their symbiotic bacteria from their intestines into the hemocoel. The bacteria multiply in the insect hemolymph, and the infected host usually dies within 24 to 48 hours. After the death of the host, nematodes continue to feed on the host tissue, mature, and reproduce. The progeny nematodes develop through four juvenile stages to the adult. Depending on the available resources, one or more generations may occur within the host cadaver, and a large number of infective juveniles are eventually released into environment to infect other hosts and continue their life cycle [39].

Name of nematodes	Host
S. glaseri	White grubs (scarabs, especially Japanese beetle, *Popillia* sp.), banana root borer
S. kraussei	Black vine weevil, *Otiorhynchus sulcatus*
S. carpocapsae	Turf grass pests—billbugs, cutworms, armyworms, sod webworms, chinch bugs, crane flies. Orchard pests, ornamental and vegetable pests—codling moth, banana moth, cranberry girdler, dogwood borer and other clearwing borer species, black vine weevil, peach tree borer, shore flies (*Scatella* spp.)
S. feltiae	Fungus gnats (*Bradysia* spp.), shore flies, western flower thrips
S. scapterisci	Mole crickets (*Scapteriscus* spp.)
S. riobrave	Citrus root weevils (Diaprepes spp.) mole crickets
H. bacteriophora	White grubs (scarabs), cutworms, black vine weevil, flea beetles, corn root worm, citrus root weevils (Diaprepes spp.)
H. megidis	Weevils
H. indica	Fungus gnats, root mealybug, grubs
H. marelatus	White grubs (scarabs), cutworms, black vine weevil
H. zealandica	Scarab grubs

(**Source:** Tofangsazi *et al* 2015) [41]

3.5 Protozoa
Entomopathogenic protozoans are extremely diverse group of organisms comprising around 1000 species attacking invertebrates including insect species and are commonly referred as microsporidians [42]. They are generally host specific and slow acting, producing chronic infections with general debilitation of the host. The spore formed by the protozoan is the infectious stage and has to be ingested by the insect host for pathogenicity. The spore germinates in the midgut and sporoplasm is released invading the target cells causing infection of the host. The infection results in reduced feeding, vigour, fecundity and longevity of the insect host as inundatively applied microbial control agents. Only few species has been moderately successful [43]. However, the utility of *N. locustae* as a grasshopper biocontrol agent remains questionable because of the great difficulty in assessing the efficacy in case of a highly mobile insect [44]. *Nosema pyrausta* is another beneficial microsporidian that reduces fecundity and longevity of the adults and also causes mortality of the larvae of European corn borer [45].

4. Conclusion
Current problems with the use of chemical insecticides and emphasis on low inputs sustainable agriculture have pushed the microbial agents to the fore front for use in IPM systems. The microorganism provides certain distinct advantages over many other control agents and methods. The major advantage of exploiting microorganism for pest control is their environmental safety primarily due to the host specificity of these pathogens. Microorganisms have natural capability of causing disease at epizootic levels due to their persistence in soil and efficient transmission. Many insect pathogens are compatible with other control methods including chemical insecticides and parasitoids. The cost of development and registration of microbial insecticides is much less than that of chemical insecticides. There is a minimum effect on non-target organism. There is a long term regulation of a pest population in most the cases whereas in others fairly a good check of pest population has been established. The large scale culture and application is relatively easy and inexpensive. The self-perpetuating nature of most of the pathogens in both space and time would certainly prove to be an asset in sustainable agriculture.

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