

Full Length Research

A Review of the Microbial Biopesticides: Types and Applications

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Abstract: The aim of this study is to carry out a detailed review of the literature on microbial pesticides, types and applications. This is because the term microbial biopesticides is a broad array from micro-organisms and other natural sources, and processes involving the genetic incorporation of DNA into agricultural commodities that confer protection against pest damage (plant-incorporated protectants). Some microbial pesticides, such as *Bacillus thuringiensis*, have a long history of safe and effective use as a biological insecticide. More recent developments in microbial pest control include the utilization of other bacterial and fungal species that may competitively inhibit the growth of pathogenic and toxigenic micro-organisms on important agricultural commodities. The study adopts an extensive review of literature such as conference papers, journal articles, internet sources, books to find out the effects of microbial biopesticides, types and applications as reported by past authors with the help of standard literature procedures in their research work. These secondary sources of information were reviewed to understand the microbial biopesticides, types and applications. Authors in this study found that the use of microbes and their gene products introduces additional considerations to the toxicological dose-response relationship, including a need to determine the plausibility of infectious and immunological effects in association with human exposure to these biopesticides in food or the environment. Studies of substantial equivalence suggest that foods currently derived from plant-incorporated protectants are not likely to differ from conventional foods. However, there is general consensus that the scientific methods to assess risks from genetically modified foods and micro-organisms will continue to evolve in the future.

Keywords: Microbial Biopesticides: Types: Applications: Pest Control

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1.0 Introduction of the Study

Microbial Biopesticides refers to the action of parasites, predators or pathogens, on a pest population which reduces its numbers below a level causing economic injury. Microbial biopesticides contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient; each active ingredient is relatively specific for its target pest(s) (Nasrine, 2013; Okhani, 2018). Microbial pesticides, is one of three major classes of biopesticides, are composed of naturally occurring bacteria, viruses, fungi, or protozoans that target a specific problem. However, there are several types of microbial biopesticides available that can be used to eliminate several different types of pests (Opende, 2011; Ukonu et al., 2022b; Owolabi et al., 2022). Food losses in the world are

high. The main aspect of this problem is the due to damage of crops that leads to loss of production and this also affects the health of humans (Shilpi & Promila, 2012). The damage and destruction inflicted on crops by pests have had a serious impact on farming and agricultural practices for a long time. These pests include insects, fungi, weeds, viruses, nematodes, animals and birds. It has been estimated that nearly 10 000 species of insects, 50 000 species of fungi, 1800 species of weeds and 15 000 species of nematodes destroy food and fibre crops used by millions of people worldwide (Opender, 2011). However, the very properties that give these chemicals useful-long residual action and high toxicity for a wide spectrum of organisms, have given rise to serious environmental problems. Furthermore, the emergence and spread of increasing resistance in many vector species, concerns over environmental pollution, human health and the ever increasing cost of the new chemical insecticides, with subsequent impact on the food chain ground water contamination make it apparent that vector and pest control can no longer be safely based upon the use of chemicals alone (Nasrine, 2013; Opender, 2011).

Fortunately, most insect pests have pathogenic microorganisms associated with them. Entomopathogens have been suggested as controlling agents of insect pests for over a century, and belong to species of fungi, viruses, bacteria, and protozoa. Insect pathology per se probably had its beginning in the nineteenth century under the stimulus of Bassi and Pasteur. A significant contribution to microbial control of insects was made by Mechnikoff in 1879 & Krassilnikow in 1888, who were the first to document that an entomopathogen, a muscardine fungus, *Metarrhizium anisopliae* could be mass produced and applied as a microbial insecticide to control the grain and the sugar beet pests. The control of insect pests with bacteria was probably first attempted by d'Herelle in 1914, approximately 35 years after Pasteur's description of silkworm diseases. Apparently the control was not consistent and therefore interest in bacterial pathogens was curtailed (Nasrine, 2013; Okhani, 2018). However, after a lag period of nearly 30 years, White and Dutky succeeded in 1940 in demonstrating a control of the Japanese beetle by distributing spores of the milky disease bacterium *Bacillus popilliae*. This success stimulated further investigations of bacteria and literature began appearing on the effectiveness of *Bacillus thuringiensis*. The issuing of eight patents between 1960 and 1963 for *B.thuringiensis* led to a revived interest in bacterial insecticides. The use of viruses to control insect pests was stimulated by the studies of Balch and Bird in 1944 and Steinhaus and Thompson in 1949, respectively. This initial interest is presently having a rebirth, as is evidenced by the recent registration of the first viral pesticide in the United States by the Environmental Protection Agency (EPA) (Nasrine, 2013). Biological control methods can be used as part of an overall integrated pest management program to reduce the legal, environmental, and public safety hazards of chemicals. In addition, it may be a more economical alternative to some insecticides. Unlike most insecticides, biological controls are often very specific for a particular pest. There is less danger of impact on the environment and water quality and they offer a more environmentally friendly alternative to chemical insecticides. They could also be used where pests have developed resistance to conventional pesticides (Nasrine, 2013).

2.0 Review of Literature

2.1 Microbial Biopesticides

Microbial biopesticides represent an important option for the management of plant disease. Microbial insect control utilizes pathogenic microorganisms isolated from diseased insects during naturally occurring epidemics (Shilpi & Promila, 2012). Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or algae) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that kill specific insects. They suppress pest by producing a toxin specific to the pest, causing disease, preventing establishment of other microorganisms through competition or other modes of actions (Shilpi & Promila, 2012). Microbial control agents, based on naturally occurring microbes have offered some realistic alternatives to chemical pesticides when used as part of an ecologically based integrated pest management (EBIPM) or area-wide pest management strategy (AWPM) (Koul et al., 2007). Out of all the biopesticides used today, microbial biopesticides constitute the largest group of broad-spectrum biopesticides, which are pest specific (i.e., do not target non-pest species and are environmentally benign). There are at least 1500 naturally occurring insect-specific microorganisms, 100 of which are insecticidal (Khahatourians, 2009). Over 200 microbial biopesticides are available in 30 countries affiliated to the Organization for Economic Co-operation and Development (OECD) (Kabaluk & Gazdik, 2007). There are 53 microbial biopesticides registered in the USA, 22 in Canada and 21 in the European Union (EU) (Kiewnick, 2007), although reports of the products registered for use in Asia are variable (Thakor, 2006). Overall, microbial biopesticide registrations are increasing globally, the expansion of various technologies has increased the scope for more products and the change in the trend to develop microbial products is definitely on the rise (Bailey, 2010). There are many reasons for the recent increased interest in microbial biopesticides, including the development of resistance to conventional synthetic pesticides, a decline in the rate of discovery of novel insecticides, increased public perception of the dangers associated with synthetic pesticides, host-specificity of microbial pesticides and improvement in the production and formulation technology of microbial biopesticides (Opender, 2011).

2.2 Bacterial Biopesticides

Four (4) categories of bacteria are used as biopesticides: Crystalliferous spore formers (such as *Bacillus thuringiensis*); Obligate pathogens (such as *Bacillus popillia*); Potential pathogens (such as *Serratia marcescens*) and Facultative pathogens (such as *Pseudomonas aeruginosa*). The spore formers have been most widely adopted for commercial use because of their safety and effectiveness (Roh & Choi, 2007; Shehu et al., 2022; Ukonu et al., 2022a). The most commonly used bacteria are *Bacillus thuringiensis* and *Bacillus sphaericus*. *Bacillus thuringiensis* is a specific, safe and effective tool for insect control (Roh & Choi, 2007). It is a Gram-positive, spore-forming, facultative bacterium, with nearly 100 subspecies and varieties divided into 70 serotypes (Schnepf, 2002). Subspecies of *Bacillus thuringiensis* that are used as biopesticides include *Bacillus thuringiensis tenebrionis* (targeting Colorado potato beetle and elm leaf beetle larvae), *Bacillus thuringiensis kurstaki* (targeting variety of caterpillars), *Bacillus thuringiensis israelensis* (targeting mosquito, black fly and fungus gnat larvae) *Bacillus thuringiensis aizawai* (targeting wax moth larvae and various caterpillars, especially the diamond and black moth caterpillar). These bacteria are mass produced through either solid or liquid fermentation. The loss of one litre of medium for the production of *Bacillus thuringiensis israelensis* has been estimated as US\$ 1.2 and 0.01 using commercial complex medium versus by-products of industrial factories, respectively. Some common commercial products based on *Bacillus thuringiensis* are available globally (Opender, 2011). *Bacillus sphaericus* is a Gram-positive strict aerobic bacterium, which produces round spores in a swollen club-like terminal or subterminal sporangium (Park, 2010). *Bacillus sphaericus* strains were isolated in the mid-1960s from mosquitoes, blackflies and grasshoppers (Berry, 2009). *Bacillus sphaericus* based products are commonly used for mosquito control. Other species of bacteria have little impact on pest management, through some commercial products based on *Agrobacterium radiobacter*, *Bacillus popilliae*, *Bacillus subtilis*, *Pseudomonas cepacia*, *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Pseudomonas solanacearum* and *Pseudomonas springaenare* available (Opender, 2011).

2.3 Mechanisms of Action of Bacterial Biopesticides

When this parasporal crystal is ingested by the largest insects, the protoxin gets activated within its gut by a combination of alkaline pH (7.5 to 8.5) (De Barjac, 2009). The active form of the toxin protein gets itself inserted into the membrane of the gut epithelial cells of the insects, this results in the formation of ion channels. There occurs an excessive loss of cellular ATP, as a consequence cellular metabolism ceases, insects stop feeding and become dehydrated (Khachatourians, 2009). Bacterial toxin opens certain selective pores in the membrane leading to the inflow of cation into the cells that causes osmotic lysis and destruction of epithelial cells and this induces starvation and lethal septicemia of the target pest and finally the death of the insect. (Rodrigio-Simon *et al.*, 2008; Shehu et al., 2022; Ukonu et al., 2022a).

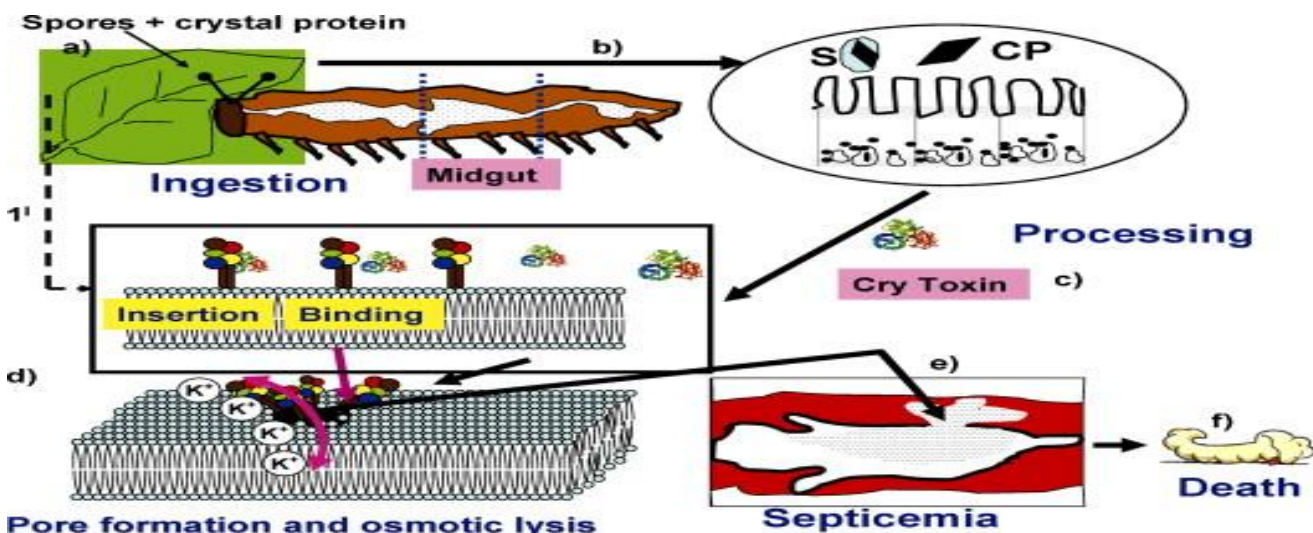


Figure 1 Mechanisms of Action of Bacterial Biopesticides

Source (Khachatourians, 2009)

2.4 Viral Biopesticides

Over 700 insect-infecting viruses have been isolated, mostly from Lepidoptera (560) followed by Hymenoptera (100), Coleoptera, Diptera and Orthoptera. (40) (Khachatourians, 2009). The viruses used for insect control are the DNA-containing baculoviruses (BVs), Nucleopolyhedrosis viruses (NPVs), granuloviruses (GVs), acoviruses, iridoviruses, parvoviruses, polydnaviruses, and

poxviruses and the RNA-containing reoviruses, cytoplasmic polyhedrosis viruses, nodaviruses, picorna-like viruses and tetraviruses. However, the main categories used in pest management have been NPVs and GVs. These viruses are widely used for control of vegetable and field crop pests globally, and are effective against plant-chewing insects. Their use has had a substantial impact in forest habitats against gypsy moths, pine sawflies, Douglas fir tussock moths and pine caterpillars. Codling moth is controlled by *Cydia pomonella* GVs on fruit trees, and potato tuberworm by *Phthorimaea operculella* GVs in stored tubers. Virus-based products are also available for cabbage moths, corn earworms, cotton leafworms and bollworms, beet armyworms, celery loopers and tobacco budworms.

The viral biopesticides are usually only active against a narrow host spectrum and after their application to plant surfaces, and baculovirus occlusion bodies (OBs) are rapidly inactivated by solar ultraviolet (UV) radiation, particularly in the UV-B range of 280-320 nm (Killick, 2009). However, their efficacy can be improved by the use of formulations that include stilbene-derived optical brighteners, which increase susceptibility to NPV infection by disrupting the peritrophic membrane (Okuno, 2003) or inhibiting sloughing (Washburn, 2012) or virus-induced apoptosis of insect midgut cells (Dougherty, 20067). UV inactivation could be controlled by creating systems, which can filter UV radiation, as has been demonstrated by using plastic greenhouse structures that reduced the intensity of incident UV-B (280-315 nm) readings by >90% compared with external readings leading to an increase in the prevalence of infection in larvae (Lasa, 2007).

2.5 Mechanism of Action of Viral Biopesticides (Viral Pathogenesis)

About a dozen of these viruses have been commercialized for use as biopesticides. The mechanism of viral pathogenesis is through replication of the virus in the nuclei or in the cytoplasm of target cells. The expression of viral proteins occurs in three phases. First is the early phase, i.e. 0-6 h postinfection, second is the late phase, i.e. 6-24 h postinfection and the third phase is very late phase, i.e. up to 72 h post-infection. It is at the late phase that virions assemble as the 29 kDa occlusion body protein is synthesized. Numerous virions of NPVs are occluded within each occlusion body to develop polyhedra. However, the GV virion is occluded in a single small occlusion body, to generate granules. Infected nuclei can produce hundreds of polyhedra and thousands of granules per cell. These can create enzootics, deplete the pest populations, and ultimately create a significant impact on the economic threshold of the pest.

2.6 Fungal Biopesticides

The pathogenic fungi are another group of microbial pest management organisms (Khachatourians, 2009), that grow in both aquatic as well as terrestrial habitats and when specifically associated with insects are known as entomopathogenic fungi. These are obligate or facultative, commensals or symbionts of insects. The pathogenic action depends on contact and they infect and kill sucking insect pests such as aphids, thrips, mealy bugs, whiteflies, scale insects, mosquitoes and all types of mites (Barbara, 2003). Entomopathogenic fungi are promising microbial biopesticides that have a multiplicity of mechanisms for pathogenesis. They belong to 12 classes within six phyla and belong to four major groups; Laboulbeniales, Pyrenomycetes, Hyphomycetes and Zygomycetes. Some of the most widely used species include *Beauveria bassiana*, *Metarhizium anisopilae*, *Nomuraea rileyi*, *Paecilomyces farinosus* and *Verticillium lecanii*. Many of them have been commercialized globally (Sparks, 2001). Spinosyns are commercially available biopesticidal compounds that were originally isolated from the actinomycete *Saccharopolyspora spinosa* (Sparks, 2001), and are active against dipterans, hymenopterans, siphonaterans and thysanopterans but are less active against coleopterans, aphids and nematodes (Sparks, 2001).

2.7 Mechanism of Action of Fungal Biopesticides (Fungal Toxin)

The fungi attack the host via the integument or gut epithelium and establish their conidia in the joints and the integument (Pekrul, 2009). Some species such as *B. bassiana* and *M. anisopilae* cause muscardine insect disease and after killing the host, cadavers become mummified or covered by mycelial growth (Miranpuri, 2009). Some fungi, primarily Streptomycetes, also produce toxins that act against insects. About 50 such compounds have been reported as active against various insect species belonging to Lepidoptera, Homoptera, Coleoptera, Orthoptera and mites (Cole, 2009). The most active toxins are actinomycin A, cycloheximide and novobiocin.

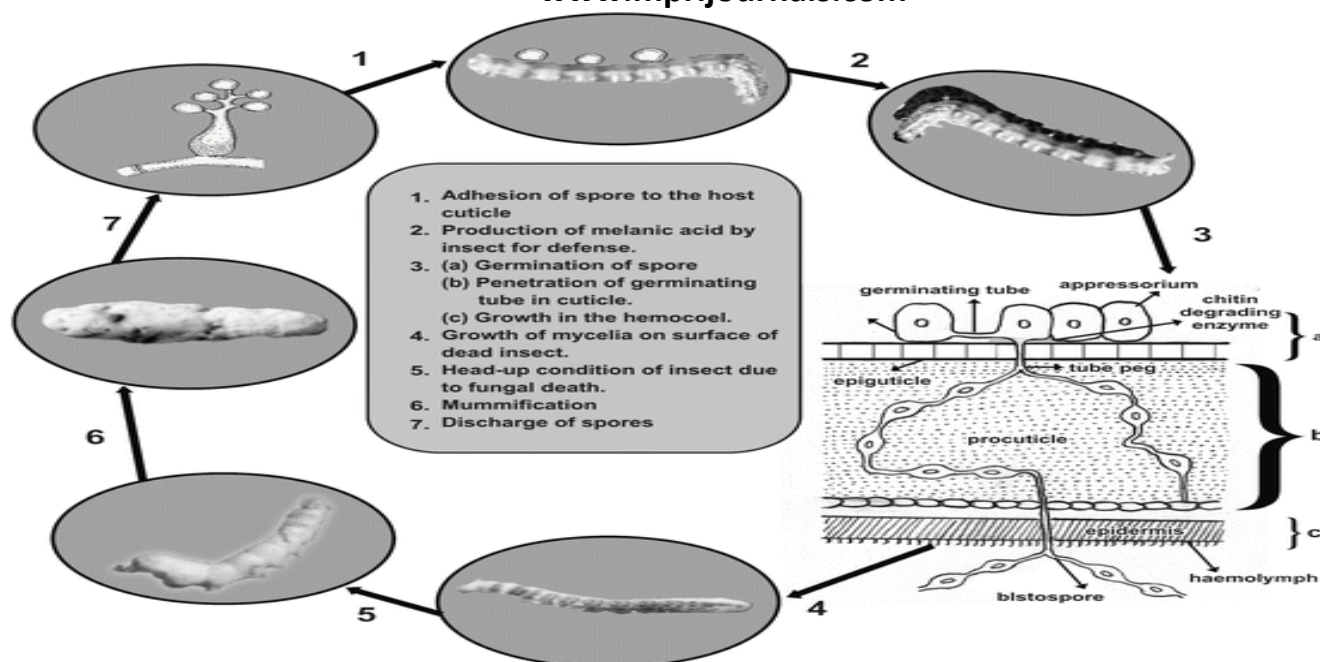


Figure 2 Mechanism of Action of Fungal Biopesticides

Source (Miranpuri, 2009)

2.8 Nematode Biopesticides

Another group of microorganisms that can control pests is the entomopathogenic nematodes, which control weevils, gnats, white grubs and various species of the Sesiidae family (Williams, 2002). These fascinating organisms suppress insects in cryptic habitats (such as soil-borne pests and stem borers). Entomopathogenic nematodes (EPN) can be mass-produced *in vivo* and *in vitro* in solid media or liquid fermentation (Shapiro-ian, 2006). Nematodes that have been successfully produced in fermenters (7500-80 000-litre capacity) include *Steinernema carpocapsae*, *S. riobrave*, *Steinernema glaseri*, *Steinernema scapterisci*, *Heterorhabditis bacteriophora* and *Heterorhabditis megidis*, with a yield capacity up to 250 000 IJS/ml (Shapiro-ian, 2006). The use of nematodes is done using a curative rather than prophylactic approach (Grewal, 2005), for instance, as demonstrated in the case of *Synanthedon exitiosa*, using *S. carpocapsae* and *H. bacteriophora* nematode species to induce field suppression of the pest in a curative manner (Shapiro-ian, 2006); 1 50 000- 300 000 Us/tree were used three times during September and October for three consecutive years in order to obtain as much control as was achieved with chemical pesticides. Some commercial products are available based on *Steinernema* and *Heterorhabditis* nematode formulations. However, extensive studies are required to optimize application parameters and develop efficient strains to achieve significant control of pests through nematodes.

2.9 Mechanism of Action of Nematode Biopesticides (Infective Juveniles)

Commonly used nematodes in pest management belong to the genera *Steinernema* and *Heterorhabditis*, which attack the hosts as infective juveniles (IJs). IJs are free-living organisms, which enter the hosts through mouth, anus, spiracles or cuticle. They are able to release their bacterial symbionts in to the haemocoel of hosts, killing the host within 24-48 h (Dowd, 2002). The nematodes can complete up to three generations within the host, after which the IJs leave the cadaver to find the new hosts (Kaya, 2003).

2.10 Protozoan Biopesticides

Although they infect a wide range of pests naturally and induce chronic and debilitating effects that reduce the target pest populations, the use of protozoan pathogens as biopesticide agents has not been very successful. Protozoa are taxonomically subdivided into several phyla, some of which contain entomogenous species. Microsporidian protozoans have been investigated extensively as possible components of integrated pest management programmes. Microsporidia are ubiquitous, obligatory intracellular parasites that are disease agents for several insect species. Two genera, *Nosema* and *Vairimorpha*, have some potential as they attack lepidopteran and orthopteran insects and seem to kill hoppers more than any other insect (Kirst, 2010). The only protozoan registered for use as a biopesticide is the microsporidian, *Nosema locustae*, which infects grasshoppers. This organism is most effective when ingested by nymphal stages of grasshoppers and kills them within three to 6 weeks post-infection (Bidochka, 2009). However, not all infected grasshoppers are killed by this protozoan infection.

2.11 Mechanism of Action of Protozoan Biopesticide (Sporaplasm Spores)

A study of *Nosema pyrausta*, a microsporidium infecting the European corn borer, *Ostrinia nubilalis*, suggests that in a horizontal transmission, a spore is eaten by a European corn borer larva, which germinates in the midgut, extrudes a polar filament and injects sporaplasm into a midgut cell. The sporaplasm reproduces and then forms more spores, which can infect other tissues. Spores in infected midgut cells are sloughed into the gut lumen and are eliminated along with faeces to the maize plant. These spores remain viable and are consumed during larval feeding so that the infection cycle is repeated in midgut cells of the new host. If a female larva is infected, *Nosema* is passed to the filial generation by vertical transmission. As the infected larva develops through to an adult the ovarial tissue and developing oocytes become infected with *N. pyrausta*. The embryo is infected within the yolk and when larvae hatch, they are infected with *N. pyrausta*. Both horizontal and vertical transmissions maintain *N. pyrausta* in natural populations of European corn borer. *N. pyrausta* suppresses populations of European corn borer by reducing oviposition, percentage hatch and survival of infected neonate larvae (Bidochka, 2009).

Table 1: Advantages and Disadvantages of Microbial Pesticides over Chemical Pesticides

Microbial Pesticides	Chemical Pesticides
<p>Advantages</p> <ul style="list-style-type: none"> ❖ Microbial pesticides are non toxic and non-pathogenic to non-target organisms and the safety offered is their greatest strength. ❖ Action of microbial is specific to a single group or series of pests, therefore, do not affect directly beneficial animal such as predators and parasitoids. ❖ Residual of microbial pesticides are non-hazardous and are safe at all time, even close to harvest periods of the crops. <p>Disadvantages</p> <ul style="list-style-type: none"> ❖ Owing to the specificity of the action, microbes may control only a portion of pests present in a field and may not control other type of pests ❖ present in treated areas, which can cause continuous damage. ❖ As heat, UV light and desiccation reduces the efficacy of microbial pesticides, the delivery systems become an important factor. 	<p>Advantages</p> <ul style="list-style-type: none"> ❖ They are easily available in large quantities, at high quality and at reasonable price. ❖ Pesticides are often used to stop the spread of pests in imports and exports, preventing weeds and protecting households from destruction. ❖ They have substantial application in protection of pets and humans from pests. <p>Disadvantages</p> <ul style="list-style-type: none"> ❖ Overuse of chemical pesticides encourages resistance. ❖ There are poisoning hazards for pesticide operators given excessive exposure; though it depends on dose, toxicity, sensitivity and duration of exposure. ❖ Drift of sprays and vapour of chemical pesticide can cause severe problems in different crops, waterways and general environment.

3.0 Methodology of the Study

The study adopts an extensive review of literature such as conference papers, journal articles, internet sources, books to find out the effects of microbial pesticides, types and applications. This is because the activities as reported by past authors with the help of standard literature procedures in their research work. These secondary sources of information were reviewed to understand as the aim of this study is to carry out a detailed review of the literature on microbial pesticides, types and applications.

4.0 Conclusion of the Study

The aim of this study is to carry out a detailed review of the literature on microbial pesticides, types and applications. This is because the term microbial biopesticides is a broad array from micro-organisms and other natural sources, and processes involving the genetic incorporation of DNA into agricultural commodities that confer protection against pest damage. Some microbial pesticides, such as *Bacillus thuringiensis*, have a long history of safe and effective use as a biological insecticide. More recent developments in microbial pest control include the utilization of other bacterial and fungal species that may competitively inhibit the growth of pathogenic and toxigenic micro-organisms on important agricultural commodities. Authors in this study found that the use of microbes and their gene products introduces additional considerations to the toxicological dose-response relationship, including a need to determine the plausibility of infectious and immunological effects in association with human exposure to these biopesticides in food or the environment. Authors also reported of substantial equivalence suggest that foods currently derived from plant-incorporated protestants are not likely to differ from conventional foods. However, there is general consensus that the scientific methods to assess risks from genetically modified foods and micro-organisms will continue to evolve in the future. Biopesticide are typically microbial biological pest control that is applied in manner similar to chemical pesticides, available in different formulations,

also used to control soil borne and seed borne fungal pathogens and more work should be done on microbial biopesticide and encourage people should use microbial biopesticide to control pests. Microbial biopesticide methods should be used as an overall integrated pest management program to reduce the legal, environmental, and public safety hazards of chemicals.

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