Microbial Insecticides- An Ecofriendly Effective Line of Attack for Insect Pests Management

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***Abstract*— This paper outlines the existing formal familiarity on the possible usage of microbial insecticides in control of global insect pests. On agricultural and horticultural crops, the several insects species are common, and economically important pests are apparently colonized by microbes called entomopathogens. Essential groups of microbes that parasitize insects are the bacteria, viruses, fungi, nematodes and protozoa, which have been used to control insect pests in the field. Microbial control of insects is achieved through the inundative application of allowable formulations of insect pathogenic bacteria (*Bacillus thuringiensis*), insect pathogenic fungi (*Beauveria bassiana*), insect viruses (nuclear polyhedrosis and granulosis), nematodes (*Steinernema* and *Heterorhabditis*) or protozoan (*Nosema locustae*). The potential benefits to agriculture and public health through the use of microbial insecticides are considerable owing to the interest based on the drawbacks associated with chemical pesticides. Microbial insecticides are available for treatment of soil, foliar and postharvest pathogens, pest nematodes, herbivorous insects, structural pests, and weeds. Any insect pathogens release metabolites, a wide array of toxins and molecules that induce changes in or modify behavior to increase the chance of insect's death. They are generally less destructive to beneficials, cause less environmental pollution and are less acutely toxic to mammals than conventional pesticides. The quality of commercially available biocontrol agents is an important consideration. The most important single requirement for the production of microbial insecticides is a supply of reproducible, reliable and authentic cultures of the microorganism. Mass production of the selected microbial agents is a necessary prerequisite for any large-scale field application, and the methodology involved has been developed at an early stage to suit a number of different pests pathogen situations. Biological and microbial control agents are living organisms, and must not be mishandled during shipping, storage, or application. Expectedly, the new research achievements in the field of microbes might announce a future microbial insecticides era, with a new generation of broad spectrum entomopathogens.**

***Index Terms*− Microbial Control, Entomopathogen, Bacteria, Viruses, Fungi, Nematodes, Protozoa**

I. INTRODUCTION

With the passage of time, pest management approach, methods and discipline have experienced developments and advancements to minimize environmental impacts. Microbial insect control utilizes pathogenic microorganisms isolated from diseased insects during naturally occurring epidemics. Typically, such epidemics only occur when pest population densities are high and usually after appreciable damage have been done to crops [1], [2], [3]. Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described including *Bacillus thuringiensis*, varieties of which are manufactured and sold throughout the world primarily for the control of caterpillar pests and more recently mosquitoes and black flies. So far, more than 40,000 species of *B. thuringiensis* have been isolated and identified belonging to 39 serotypes. These organisms are active against either Lepidoptera, Diptera or Coleoptera **pests** [4]**.**

Microbial control of pest insects is increasing in importance because of increasing resistance of arthropods to chemical insecticides, improved performance and cost-competitiveness of microbial insecticides, reduced environmental costs, and a decrease in development of new chemical insecticides [5]. As a result, an extreme caution must be exercised in using microbial insecticides. If such biological control agents are simply used as replacements for chemical insecticides i.e., inserting a microbial toxin gene into the genome of an agricultural crop, they would surely encounter similar problems to those faced by chemical insect pest control methods [6]. Infact microbial entomopathogens may not experience the potential problems faced by chemical insecticides and genetically modified organisms as they use a more dynamic system for infecting and killing host insects. However, at the present state of the art, microbial insecticides have not yet achieved their potential due to the lack of truly transformational associated technologies that may enhance their effectiveness [7].

II. IMPORTANCE OF MICROBIAL PESTICIDES

A microbial toxin can be defined as a biological poison derived from a microorganism, such as a bacterium or fungus. Pathogenesis by microbial entomopathogens occurs by invasion through the integument or gut of the insect, followed by multiplication of the pathogen resulting in the death of the host e.g., insects. Studies of natural epizootics of entomopathogenic bacteria and fungi, have stimulated man`s interest in employing them as microbial pesticides and myco-insecticides to control agricultural pests. However, this technology stagnated as disillusionment as to the practical value of virus, bacteria and fungi as biological control agents of arthropods, and their efficacy against a range of pests became dominant in various parts of the world, following the initial overwhelming success of chemical pesticides. The revival of interest in microbial insecticides over the last 20 years, has led to large-scale production of *Bacillus thuringiensis* [Berliner], and the marketing of the first bacterio-insecticides. Microbial insecticides essentially do not pose a disease risk to wildlife, humans, and other organisms not closely related to target insect. In fact, they can be applied when a fruit or vegetable is almost ready for

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harvest without the risk of killing beneficial insects in the process [8], [9], [10], [11].

III. CONSIDERING OF MICROBIAL INSECTICIDES

Single cell organisms, such as bacteria, fungi and protozoa and viruses, have been mass produced and formulated for use in a manner similar to insecticides. Products containing these organisms can be regulated by the environmental protection agency and use is governed by the federal insecticide, fungicide and rodenticide acts [12], [13].

1. *Bacillus thuringiensis* (Kurstaki)

The bacterium, *Bacillus thuringiensis* (B. t.), reproduces by spores and the spores are produced in the bacterium cell along with a crystalline protein called an endotoxin. The endotoxin, with or without the spores must be ingested by the target insect in order to be effective. Once ingested, the endotoxin is activated by the alkaline conditions in the insect's stomach. The toxin attaches to specific receptors on the gut wall, causing the gut lining to break down. This method normally allows the spores to enter the host's blood (hemolymph) where the bacterium can proliferate. Different species and strains and Bacillus bacteria are known to affect different groups of insect pests, primarily due to differences in endotoxin receptor sites on the gut wall:-

i. *Bacillus thuringiensis* var. *kurstaki*- acts on caterpillars of moths and butterflies.
ii. *Bacillus thuringiensis* var. *israelensis*- is for larvae of flies such as fungus gnats.
iii. *Bacillus thuringiensis* var. *san diego*- is useful for larvae of beetles such as elm leaf beetles and Colorado potato beetles [14], [15].

Among the wide variety of bacteria associated to insects, there are different examples of entomopathogenic species other than Bacillus that have been studied at different levels. These include, for instance, *Clostridium bifermentans serovar malaysia* that is active against mosquitoes and blackflies [16]. Another group of entomopathogenic bacteria with high interest is represented by the endosymbionts of insecticidal nematodes, especially the members of the genera Xenorhabdus and Photorhabdus. The first is associated to nematodes in the genus Steinernema, while the second colonize the intestines of Heterorhabditis species. Normally, after nematodes invade susceptible insect hosts, symbiotic bacteria are released in the hemocoel where they produce various virulence factors contributing to impair insect immune system and to kill the host. Significant is also the case of *Pseudomonas entomophila*, that is an ubiquitous bacterium showing insecticidal properties against insects in different orders and which has the capacity to trigger a systemic immune response in *Drosophila melanogaster* Meigen after ingestion [17].

2. Viruses

Insect-specific viruses can be highly effective natural controls of several caterpillar pests. Epizootics can occasionally devastate populations of some pests, especially when insect numbers are high. Insect viruses need to be eaten by an insect to cause infection but may also spread from insect to insect during mating or egg laying. In some cases, for example while searching. Baculoviruses are target specific rod-shaped viruses, which can infect and destroy a number of important plant pests. They are particularly effective against the lepidopterous pests of cotton, rice and vegetables. Their large-scale production poses certain difficulties, so their use has been limited to small areas. Nuclear polyhedrosis viruses and granulosis viruses are available to control some caterpillar pests [18]**. Virus products codling moth, *Heliothis zea* and beet armyworm nuclear polyhedrosis virus are registered for control of pest Lepidoptera, such as the cotton bollworm and cotton budworm that caterpillars are also pests of corn, soybean and other vegetables, while granulosis virus are for alfalfa looper and leaf roller** [19], [20]**.**

3. Fungi

Several insect pathogenic fungi are used as microbial control agents, including *Beauveria*, *Metarhizium* and *Paecilomyces*. These are most often used against foliar insect pests in greenhouses or other locations where humidity is relatively high. Several fungi have been studied as potential microbial insecticides. The *Beauveria bassiana* can affect a wide variety of arthropods. However, environmental conditions, particularly temperature and humidity are important factors effecting the success of fungal treatments, particularly when using preparations of fungal spores. These fungi Tolypocladium, Isaria and Lecanicillium have been applied inundatively, in the manner of a chemical application, with the goal of killing most, if not all, insects. In comparison to chemical controls, knockdown is slower and generally incomplete. However, establishment of the pathogen may result in death of insects well beyond the period attained by chemical controls. Indeed, the risks of resistance are less and the effects on non-target organisms hugely reduced. In general, when a spore comes in contact with the insect cuticle, the spore attaches, germinates, penetrates the cuticle (from outside in, or inside out, depending on whether the spore lands on the outer surface, or is ingested). Indeed, many entomopathogens are closely related to fungi known to release a wide array of toxins and molecules that induce change in or modify behaviour (Cordycipitales, Trichocomaceae etc). The fungus has since been used successfully to control the larvae of many Lepidopteran insects. Metarrhizium, also an asexual stage of an Ascomycete, has been successfully used to control spittlebugs. *The Lecanicillium lecanii* infects sucking insects such as aphids, white flies and scale insects. The fungus is used to control sucking insects or leaf mining insects mostly in greenhouses where humidity is high. Some plant-sucking aphids also have the nasty habit of transmitting viruses, thus control of the insect and the viruses it transmits are both possible. Tolypocladium and Isaria have been reported to colonize a wide range of insects. Tolypocladium is particularly interesting because it effectively controls mosquitos in experimental conditions [21], [22].

4. Protozoa

The protozoan *Nosema locustae* is available in a few products sold for the control of grasshoppers. Effectiveness of these products for small-scale use, such as gardens and yards has not been demonstrated. The spore causing disease must be ingested to be effective and it is very slow acting. Grasshoppers are strong fliers and can easily move long distances, making the effectiveness of these treatments on a small scale questionable. The protozoa subphrla, sporozoa and onidospora contain numerous entomophilic protozoans and are most promising examples in biological insect pest suppression programs. The effects of protozoan infections are chronic rather than acute and they may affect their hosts over a fairly long time period. Because of this, disease is often manifested in the host insect only by a reduction vitality, fecundity and life span. The naturally occurring epizootics of protozoan cause disease in insect pest like corn borer, some Lepidoptera, several species of flies, aquatic Diptera including mosquitoes and grasshoppers. The Neogregarires occurs primarily in the fat body and intestinal tract of Coleoptera, Lepidoptera, Hemiptera and Diptera. The *Mettasia grandis* is an important pathogen of the cotton boll weevil and shows considerable promise. The *Mettaisa frogodermae* has been studied and used in pest suppression program of khapra beetle. The infective spores are totally empty. The emerging sporozoites are motile in the gut tract and soon penetrate the gut to the haemocoel and infect cells of susceptible tissues within 2 days. The diseased larvae die early within a week and shrivel so badly that they are used for spore production. The *Nosema locustae* also attacks grasshopper or locust species and spores are applied in bran bait [23].

5. Entomopathogenic Nematodes

Most peoples think of nematodes as being plant parasites and causing symptoms similar to that of plant pathogens. However, some nematodes attack and kill insects, wherein the 'entomo' refers to insects and pathogenic, of course, means to create disease symptoms within. These nematodes do not directly kill the insect, enter the insect via natural openings such as the mouth, anus, or spiracles, carrying a bacterium. Once inside the host insect, the bacterium becomes active, nematode feeds on this bacterium, and the waste by-products of the bacterium become lethal to the insect, killing it by bacterial septicemia. Nematodes require an aqueous environment or they become inactive. Entomopathogenic nematodes work well on wood boring larvae that keep their tunnels open, thus creating a perfect environment for the nematodes (dark and moist). Such wood borers include the clear-winged borers (family: Sesiidae) like the peach tree borer and the dogwood borer. Many nematode products are labeled for soil inhabiting pests such as beetle grubs and black vine weevil larvae. However, obtaining and maintaining the correct amount of soil moisture for several days is difficult and the desired level of management may not always be achieved. It is important to keep soil moist enough to allow the nematodes to swim towards their intended target. These nematodes require a moisture film around the soil particles in order to stay active and to be mobile. But if the soil is too wet, they cannot achieve traction on the soil particles and they will float helplessly in the saturated soil, or if it is too dry they will become dormant. The insect parasitic nematodes Steinernema and Heterorhabditis, infect soil dwelling insects and occur naturally or can be purchased [24], [25], [26].

IV. Production and Commercialization of Pathogens

The practical use of microbial agents which kill insects is being carried out by many scientists throughout the world. Other alternatives to chemical pesticides are also under investigation, including novel pest control systems based on parasitic and predatory insects, predatory mites, fungi, bacteria, viruses, protozoans and nematodes for human`s benefit. However, most of these novel systems have not yet been exploited in agricultural practice on a commercial scale. This is due to the series of sometimes lengthy and sometimes expensive steps which must be carried out before new pest control systems can be offered to the growers [27], [28], [29].

1. Steps Leading to Commercialization

Once any new system has been identified and characterized in the laboratory, the following steps must be completed before it can be successfully commercialized:-

1.1. Process Development

A process must be developed which can be carried out on a large enough scale to ensure that an adequate amount of the material can be made. This process must be sufficiently reliable to provide a product which is both safe and effective. In addition, the production cost must allow manufacturers to make a profit.

1.2. Product Development

Many systems for controlling pests are successful in the laboratory; however, they may fail when tried in the greenhouse or field. This is one of the many problems which must be solved by product development. Products must be manufactured and formulated in a way which makes them stable for the longest possible time, as convenient as possible to use, and as immune as possible to 'use and abuse' i.e., the failure of many peoples to store or use the product as directed and then to blame the product for poor performance. Recommendations must be developed for the use of the product in actual agricultural practice. Normally these recommendations cannot be so novel or so complex or require such unconventional equipment that peoples refuse to use the product. Often the recommendations are developed in collaboration with the appropriate advisory service to ensure that they will be accepted by the trade. For novel products, testing is necessary to establish that they are safe to use; in addition, quality control tests must be devised which will ensure that every batch will be safe and effective. Once any testing is completed and quality control protocols developed, the appropriate government authority must be approached for permission to sell the product; at present, this must be done individually for every country in the world. Once a product has been sold, it is often helpful to visit growers who have used it, either to confirm its success or if it has been failed, to investigate and determine the cause. Further development work can then be carried out to find ways of avoiding product failure in future.

2. Process of Development and Production

Before one can sell a live micro-organism as a pest control agent, a reliable method of production must be developed which yields large quantities for which a product specification can be drawn up. While, the production of bacterio-insecticides is common worldwide, there is little information available on the biotechnology of entomopathogenic fungi, and their industrial production is still relatively unsophisticated.

2.1. Organism Storage.

The first problem in producing a micro-organism is storing it in a way that ensures the retention of desirable features. The most obvious feature to be retained is pathogenicity and the second is productivity in terms of yield in the commercial production process. This is typically measured either by total biomass, or by number of infective propagules i.e., spores or fragments of mycelium, produced per litre-hour of fermentation time. Many microorganisms are known to lose desirable features either on storage or after repeated sub-culturing. Among insect pathogens, *Beauveria bassiana* has been reported to have reduced virulence after sub-culturing, whereas both *Verticillium lecanii* and *Metarhizium anisopliae* are reported as being undiminished in virulence after many passages. Organisms which lose virulence may sometimes be restored to their former potency by passing them through their normal host i.e., the target insect; however, such a technique would be cumbersome as a routine part of a production process and always presents the risk of contamination. Therefore, the problem is often avoided by storing a large number of elements of a single spore isolate in a deep frozen or freeze-dried condition. Samples are checked periodically to make certain that virulence and productivity have not diminished with time.

2.2. Fermentation Method

The standard method of production of microorganisms is the process of fermentation. There are many types of fermentation; the two most common are ‘submerged’ and ‘semi-solid’. Submerged or deep-tank fermentation is, as the name implies, a growth of micro-organisms in a fully liquid system. There are a number of advantages to fully liquid systems which include the ability to hold temperature and pH constant, the ability to pump large quantities of air into the system and disperse it by means of stirring impellers, and the ability to generate reasonably homogeneous conditions to maximize the growth of micro-organism. Despite the many advantages of submerged fermentation some fungi will not yield a satisfactory product by this technique. Semi-solid fermentation offers an alternative in which the fungi grow primarily on the wet surface of a solid material, often some form of processed cereal grain to which nutritional adjuvants have been added, though attempts are made frequently to use ‘waste’ materials or media of low value, such as straw. This allows fungi to grow in conditions more similar to those found in nature, and spores that are the infective propagules by which the fungus survives and infects insects, are produced in the air and are consequently more durable. Semi solid fermentations are relatively easy to develop on a small scale. Scaling them up to the sizes necessary for commercial product presents numerous problems; aeration becomes a major difficulty as the volume of a semi-solid mass increases more rapidly than the available surface area. This requires either a very large area of relatively shallow media e.g., on trays, or in a vessel which can agitate or tumble the media. On any scale, trays are very difficult to sterilize and keep sterile. The development of large vessels for semi-solid media fermentation requires the invention of a number of techniques or pieces of equipment for keeping the media friable after sterilization and its tendency is to set solid when it cools, rather like oatmeal, inoculation with the desired fungus without contamination, aeration and agitation during fermentation, and drying the material prior to opening the fermenter in order to avoid contamination, etc.

2.3. Medium Development

Whichever type of fermentation is chosen, nutrients must be provided so that the micro-organism can grow. Which nutrients are chosen will markedly affect how fast the organism grows, how much is produced, and often how infective the final product is. Nutrients to be provided include a carbon source e.g., glucose or molasses, nitrogen source e.g., soybean meal or yeast extract, and a ‘defined’, in which case the precise nature and quantity of every nutrient is known, or alternatively, they can contain ingredients of an indeterminate and occasionally variable nature e.g., molasses, and most commercial media are latter type.

2.4. Small Scale Production

The first task in small scale production is the development of a basic culture procedure affording reproducible results. Absolute production yields are of secondary importance, the main emphasis being put on the expected standard course of the microbial process with the attendant changes in growth, pH, consumption of nutrients, production of metabolites, and in the physiological state of the microorganisms. On attaining reproducible results, the search for individual optimum conditions can begin. The first factors to be explored are usually aeration and stirring, and next to be examined is the effect of changes in the ratio of individual components of the nutrient medium, with special reference to the source of carbon and nitrogen and their mutual proportions. The effect of different types and amounts of antifoam agents is also studied since the physical action of these agents can affect the dispersion of air in the culture and respiration of the microorganisms; some antifoam agents (vegetable oils, lard oil) can be utilized by the microorganisms, thus changing the metabolism of the culture. Fluid from laboratory and small scale fermenters can be used for tentative isolation of metabolites and preliminary tests of their quality.

2.5. Problems of Contamination of Microbial Processes

Microbial insecticides are more difficult to produce than chemical insecticides, as they require specialized substrates for cultivation, or even living host insects, therefore may cost more to produce. Microbial contamination of fermentation processes and principles and techniques for its elimination should be appraised from several viewpoints such as type of the metabolite produced, specific properties of the culture, machinery and equipment used in the process, nutrient medium and the raw materials used for its preparation, and technology adopted in the process. Contaminating microorganisms affect negatively the microbial process by destroying the cells of the production strain, inactivating the synthesized metabolites, producing substances affecting the producer`s metabolism and thus decreasing the production of the required metabolite, and exhausting compounds crucial for growth and product synthesis from the medium. However, the presence of a contaminating microorganism need not always results in a drop in metabolite or spores production; in the absence of this drop, it is often difficult to detect contamination. In semi-solid fermentation process, contamination need not always leads to failure. The term 'protected fermentation' is used for such processes in which foreign contamination is suppressed by the presence of an antimicrobial agent and this agent is added to the medium in suitable form [30], [31].

V. Approaches to Microbial Control

Microbial insecticides battle against damaging insects and these are 'unconventional' insecticides, but they can be applied in conventional ways such as sprays, dusts, or granules. Microbial control can be natural conservation of natural enemies or applied inoculation or inundation. Inoculation and inundation involve the supplemental release of natural enemies to build populations of beneficial organisms. Many biological and microbial control agents are commercially available for purchase that can be applied through conserving of existing field populations. An inoculative approach involves the release of natural enemies at a critical time of the season to augment natural populations already present, but in numbers too low for effective pest managing. An inundative approach involves the application of a large number of organisms much in same manner as a pesticide. These insecticides also may be delivered through many different media including liquids which may be serving as drenches, sprays or solids including clay-based powders or baits interlaced with microbes [32], [33], [34], [35].

VI. CONCLUSION

The recent achievements of the scientific community working in the area of insect pathogens are contributing to increase the effort directed toward the discovery of new microbe based insecticides. As with all biological control agents, it is especially important to match the correct microbial control agent with the correct pest in order for them to be effective. In this scenario the interest microbial insecticides is significantly growing, also as a result of the withdrawal of many synthetic pesticides and the high cost for the development of new ones Microbial pesticides usage in combination or rotation with synthetic pesticides is likely to be enhanced in the near future, but more research is needed to come up with innovative solutions that can really meet farmers and regulator needs in terms of effectiveness and environmental sustainability. **Microbial pesticides** consist of a microorganism (e.g., a bacterium, fungus, virus nematode or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pests. For example, there are fungi that control certain weeds, and other fungi that kill specific insects. The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bacillus control moth larvae found on plants, other Bacillus are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bacillus produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve. Microbial insecticides may provide a satisfactory alternative to chemical pesticides when used as part of an overall Integrated Pest Management (IPM) plan. **Microbial pesticides** being specific to target pests, relatively safe to non-target organisms including humans, and residue free commodities are presumed to be adoption by farmers for maximizing advantages.

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Author's Profile

1. Dr. Muhammad Sarwar, Principal Scientist, is going through 25th years of Service experience in Research orientated Department of Agriculture (16-05-1991 to 31-05-2001, Government of Punjab), and Pakistan Atomic Energy Commission (01-06-2001 to date).
2. Have 172 publications in National (60) and Foreign (112) Journals with suitable Impact Factor.
3. Award of Higher Education Commission of Pakistan "Post-Doctoral Scholarship-2006" for Post Doc., research work at Chinese Academy of Agricultural Sciences, Beijing, China.
4. Shield award, Letters of Appreciation, and Certificates of performance and honor granted from Chinese Academy of Agricultural Sciences, Beijing, China.
5. Awarded Gold Medal-2010 by Zoological Society of Pakistan (International) in recognition of research contributions in the field of Insect Science.
6. Granted Research Productivity Award-2011, by Pakistan Council for Science and Technology.
7. Included in Panel of approved Supervisor of Higher Education Commission **(**HEC), Pakistan.
8. Completed "Basic Management Course" organized by Pakistan Institute of Engineering & Applied Sciences (PIEAS), Islamabad, held from 31 January to 18 February, 2011.