Journal of Biopestcides and Environment
ISSN: 2449-1284 (Online); 2449-1292 (Print)
http://www.biopjournal.com

A REVIEW OF ENTOMOPATHOGENIC PESTICIDES AS BIOCONTROL AGENTS.

***[1]Magaji, B. T., [1]Kwanashie, A. J., [1]Utono, I., and [2]Mani, U.**
1. Department of Crop Protection,Faculty of Agriculture, Ahmadu Bello University, Zaria-Nigeria
2. Samaru College of Agriculture, Ahmadu Bello University, Zaria
*Corresponding author's e-mail: btmagaji4u@yahoo.com; Tel.: +234(0)7058888078.

ABSTRACT

In spite many folds of increase in the use of insecticide since 1940, crop losses due to insect pests have nearly doubled within the same period because, while providing effective control, the overuse or misuse of chemical pesticides have generated negative impacts on soil and water quality, human health, wildlife, increased resistance and resurgence of major insects species as well as lost of ecological balance within agro-ecosystems. This ecological concern has called for a paradigm shift both in research and practice for more eco-friendly pest management methods especially in Nigeria and other developing countries where the vicious cycle of hunger and poverty is endemic. The appropriate use of entomopathogenic pesticides solitarily or in pest management programmes have proved to be successful in suppressing the population of some agricultural insect pests and vectors of diseases. These pesticides consist of some microbes of fungal, bacterial, nematodes and viral origin as active ingredients which are perceived to be highly pest specific, long-lasting and having less potential for damage to the environment or non-target organisms. The development and production of these biocontrol agents in commercial quantities is ongoing in many parts of the developed world but its awareness and use is still limited in developing countries like Nigeria, therefore it is important for stakeholders in the agricultural sector to synergize efforts towards the development and formulation of strains that will be effective against specific insect pests in our own agro-ecological zones.

Key words: Pesticides, entomopathogenic, eco-friendly, microbes.

1. INTRODUCTION

It has been estimated that by 2050 the world population will be nine (9) billion as against its present status of over six (6) billion and that the exponential growth will occur more in the cities of developing countries, principally Africa and Asia (Rege, 2007) where food insecurity as well as child mortality due to malnutrition is endemic. Nigerian population is currently estimated to be over one hundred and fifty million (150,000,000) with an annual growth rate of about 2.5 percent (NPC, 2006). To feed this burgeoning population, produce more food and generate livelihood opportunities from less per capita arable land and water for the ever-growing population. To achieve this (food sufficiency), several agricultural programmes and policies have been developed by past government over time. Such progrmmes as green revolution, MAMSER, FADAMA proects and the rest were to empower the citizenry and at the same time ensure food availability across seasons in all parts of the country. However, this effort is been marred by a vast array of insect pests and diseases which often attack arable crops leading to decline productivity. About 67,000 field and store insect pests species are reported to cause damage to agricultural crops (Kumar, 2012) resulting to bout 42% total crop loss.

A quick and sure way of minimizing the menace of insect pests' damage to crops is through the use of conventional pesticides which are valued for their effectiveness, long shelf-life and the ease with which they are transported, stored and applied. However, the over dependence on chemical pesticides and eventual unrestrained use of them has necessitated the need for cost-effective and environmental-friendly alternative. The high cost of these pesticides coupled with pesticide misuse, fear of toxic residues in food, environmental pollution and degradation, effect on non-target organisms as well as pest's resistance and resurgence due to continuous use, has called for a paradigm shift both in research and practice in the use of more eco-friendly methods that can reduce the harmful effects of these chemical pesticides on man and his environment (Roessler 1989; Cabras *et al*., 1997).

The appropriate use of eco-friendly entomopathogenic pesticides as biocontrol agents have been reported to play a significant role in sustainable crop production by providing a stable pest management program. It is generally perceived to be long-lasting and less damaging to the environment and/or non-target organisms

The development and production of these biocontrol agents in commercial quantities is ongoing in many parts of the developed world (Khachatourians, 1986) and are being incorporated into many integrated pest management programmes (IPM) to effectively reduce pest population densities. For instance, China (Feng *et al.,* 1994) and America (Alves and Pereira, 1989) are now supplying fungal biocontrol agents in sufficient quantities for niche markets in their immediate area. These entomopathogenic pesticides consist of some microbes of fungal, bacterial, nematodes and viral origin as active ingredients. They are used solitary or in association with some compatible insecticide products to increase control efficiency.With this, the insecticide load of the environment is reduced and hence a reduction in the environmental pollution and insecticides resistance (Moino and Alves, 1998; Quintela and McCoy, 1998).

1.2 Fungi

Fungi are a diverse group of organisms found in almost every environment on earth (ubiquitous). Most have complex life cycles and some are parasitic to various eukaryotes including plants and insects. Some species have proven useful as microbial pesticides and have the ability to kill insect by attacking or infecting it (entomopathogenic fungi) (Singkaravanit *et al.*, 2010). The main route of entrance is the integument. Other routes of infection include, mouth by ingestion, wounds or trachea. Entomopathogenic fungi has an important position among all the biocontrol agents because of its route of pathogenicity, broad host range and its ability to control both sap sucking and mandibulate insect pests. They could directly breach the cuticle to invade the insect hemocoel unlike other microorganisms which only gain access into the pest by ingestion through mouth and then cause disease (Khan, *et al.,* 2012). Upon contact with insects, the fungi forms appresoria and infection peg on the cuticle, penetrates into the insect, proliferates, produces toxins and ultimately kill the insect. Following invasion, fungal pathogenesis starts with the secretion of cuticle degrading enzymes such as chitinase, protease and lipase which degrades chitin, protein and lipid which are parts of the cuticle, respectively. The spores are disseminated by the insect cadaver, air, soil and water but require high relative humidity, temperature and sunlight to complete their course of development. Most entomopathogenic fungi species are members of the fungal divisions *Ascomycota*, z*ygomycota* and the d*euteromycota.*

The most important insect infecting species occur in the genera *Aspergillus, Metarhizium, Hirsutella, Beauveria, Aschersonia, Culicinomyces, Lecanicillium, Paecilomyces, Tolypocladium* and *Sorosporella* but *Beauveria bassiana*, *Metarhizium anisopliae. Verticillium lecanii* and *Isaria fumosorosea* are currently been produced and used in commercial quantities in several countries including the United Kingdom and the United States ((Goettel *et al*., 2005). They are characterized and selected based on their insect-specific virulence properties and are capable of controlling an array of insect pests of crops such as caterpillars, borers, grasshoppers, whiteflies, aphids, ants, termites, wasps and many others. For instance, *Beauveria bassiana* (Balsamo) has been found to be effective against *Spodoptera litura* (Gopalakrishnan and Narayanan, 1989), the sweet potato weevil, *Cylas formicarius*, the termite *Odontotermis brunneus* and *O. obesus* (Khader Khan *et al.,* 1993).

Beauveria bassiana has also been reported to affect the survival of the larvae of diamondback moth, *Plutella xylostella* (Vandenberg *et al.*, 1998) and cause population decline of the grasshopper, *Melanoplus sanguinipes* (Fabricius) (Askary *et al.*, 1998). In Colombia, *B. bassiana* has been used to control coffee berry borer (Valencia and Hachatourians, 1998) and *Verticillium lecanii* (Zimm. Viegas) has been found to be effective against aphids and plant pathogenic fungi like powdery mildews (Askary *et al.*, 1998; Dik *et al.,* 1998).

1.3 Bacteria

Bacteria are the most promising entomopathogenic agents of microbial pesticides. Most species belongs to the families Pseudomonadaceae, Enterobacteriaceae, Lactobacillaceae, Micrococcaseae and Bacillaceae but majority of the commercial strains belong to the genus *Bacillus. Bacillus thuringiensis* (Bt) is the most widely used. It has over 22 serotypes determined by flagellar antigen

(Merdan,1991) and accounts for approximately 90% of the world microbial pesticide market (Chattopadhyay *et. al*., 2004). Bacterial pesticides are typically used as insecticides to control insect pests and can affect approximately 525 insects belonging to different orders and families (Thakore, 2006). *B. thuringiensis* works essentially as a particulate stomach poison. In the stomach of the invaded insect, the bacteria produces an endotoxin which binds to the intestinal lining creating pores therein. This causes paralyses of the digestive system and eventually, results in death. For effective action, application of B. thuringiensis must be made on areas (upper and under leaf, fruiting bodies and leafwhorls) of the crop where the target insect feeds or be delivered where target insects can be attracted to (Pasqualini, 1993).

Each strain of the bacterium produces a different mix of proteins which specifically kills one or a few related species of insect larvae. *B. thuringiensis* is primarily used to control lepidopterous pests (moths and butterflies) but can also be used against a broad range of other pests including specific species of mosquitoes, flies, and beetles as well as a good source of pests resistance in genetically modified crops (i.e. scientists can take the gene for the *B. thuringiensis* pesticidal protein and introduce it into the plants genome. Then the plant, instead of producing the *B. thuringiensis* rather manufactures substances that destroy the pest. In Italy, two *B. thuringiensis* based preparations namely, Bt subs. kurstaki (B.t.k) and Bt isrealensis (B.t.i) are registered and used for the control of several species of Lepidoptera and Diptera respectively.

B.t.k. was particularly useful against different species of leaf-rollers which affect pear and apple as well as other lepidopterous pests that attack vineyard, cabbage, radish and maize while B.t.i. was successful against several species of mosquitoes (Pasqualini, 1993). Similarly, populations of cotton leafworm *Spodoptera littoralis* larvae on cotton and diamond back moth *Plutella xylostella* on cruciferous plants have been controlled using B.t formulations in Egypt. In the United States of America, Bt. Products have been registered for use against 23 insect pests on 20 different agricultural crops many decades ago (Falcon, 1971) and today, a good Bt. spray is been used in the control of *Heliothis* and *Helicoverpa* species on cotton and maize plants in the United States.

1.4 Viruses:

Unlike other microbial pesticides, viruses are not considered living organisms, but rather parasitically replicating microscopic elements (US EPA Fact Sheets, 2008). They are extremely small and are composed primarily of double-stranded DNA required for the virus to establish itself and reproduce. Because this genetic material is easily destroyed by exposure to sunlight or by conditions in the host's gut, an infective viral particle (virion) is protected by protein coat called a polyhedron (D'Amico, 2007). Two main classes of viruses (Granulosis and Nucleopolyhedrosis viruses) of the group baculoviruses are primarily pathogenic to insect larvae because adult insects are not susceptible to them. The insect larvae, while feeding on the plant foliage, accidentally feed upon the polyhedra which then get solubilized in the insect's midgut, thereby releasing the virions. These virions replicate within the nuclei of epithelial cells lining the midgut to produce more virions which are released in a budded form by 10–12 h pi or get occluded within the polyhedra late in the infection process. Tissue liquification and then rupture of these cells upon death of the infected larvae liberates masses of these polyhedra in the soil environment. From here, they are again ready to be ingested and infect their hosts (Mishra, 1998). All types of baculoviruses must be eaten by the host to produce an infection and each strain has a specific insect species as target. The Nucleopolyhedrosis viruses have a relatively wide range of target pests among three different insect orders, including butterflies and moths (Lepidoptera), ants, bees, and wasps (Hymenoptera), and flies (Diptera) while Granulosis viruses are limited to species of Lepidoptera (Thakore, 2006). *Cydia pomonella* of the Granulosis viruses is applied as a foliar spray onto eggs prior to hatching to control codling moth on fruit trees such as pears and apples. The larvae ingest the virus while eating the shells after hatching, get infected and die. *Cydia pomonella* granulosis virus is used typically in rotation with other control measures in both organic and conventional agriculture, and can be used in conjunction with mating disruption. It can reduce or eliminate use of organophosphates and pyrethroids for conventional agriculture and protect against pest resistance to Spinosad for organic agriculture

(Mishra, 1998). In Honduras and Mexico, the nucleopolyhedrovirus was used to cause significant mortality on *Spodoptera frugiperda* larvae- a major insect pest of maize. The highest application rates of the virus resulted to 40% mortality but decreased with time due to uv light effect. Viral bacteriophages (a virus that infects bacterial cell walls) can also be used as biopesticides. For instance, if the virus attacks bacteria that cause plant disease, it can be used as a pesticide to replace conventional products like copper or antibiotics such as streptomycin.

2. OTHER MICROBIAL PESTICIDES

2.1 *Yeast:* Some non-pathogenic antagonistic yeast species that naturally occur in plants tissue and in water isolates from a variety of crops have been developed and used as effective biopesticides for the control of post- harvest fruit rot and/or stimulate the plant's immune system. For example, *Pichia anomala* and *Candida olephila* Strain O, isolated from golden delicious apples, has been developed into an effective bio-pesticide for the control of spoilage fungi during storage of plant-derived produce. Application of this biopesticide is done immediately after harvest but before storage to pre-colonize plant wound sites and become antagonistic to particular fungal pathogens such as gray mold (*Batrytis cinerea*) and blue mold (*Penicillium expansum*) which cause post-harvest decay. However, there is evidence that some yeast produce enzymes that can degrade fungal cell walls and stimulate plant host defense pathways in freshly harvested fruits. Yeast extract hydrolysate from *Saccharomyces cerevisiae* - a common food flavor has also been found to prevent diseases such as bacterial leaf spot of tomato, post-bloom fruit drop and greasy spot disease of citrus. It enhances the natural defense mechanisms of treated plants and improves growth, yield, and shelf life of crops.

2.2 *Nematodes*: Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. They occur naturally in soil environments and locate their host in response to carbon dioxide, vibration and other chemical cues (Kaya and Gaugler 1993). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs (Grewal *et al.* 2005) for over 200 species of soil dwelling insects pest/any larvae or grubs in the soil. Entomopathogenic nematodes do not kill the insect *per se*; rather they act like a Trojan Horse, releasing a lethal bacterial weapon once they get into the insect's blood.

The infective juvenile stage (IJ) is the only free living stage of entomopathogenic nematodes. The juvenile stage penetrates the host insect via the spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle, and then enters into the hemocoel (Bedding and Molyneux 1982). Both *Heterorhabditis* and *Steinernema* are mutualistically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively (Kaya and Gaugler 1993). 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 death of the host, nematodes continue to feed on the host tissue. The nematodes develop through four juvenile stages to the adult, and then reproduce. Depending on the available resources one or more generations may occur within the host cadaver and a large number of juveniles are eventually released into environment to infect other hosts and continue their life cycle (Kaya and Gaugler 1993).

The insect cadaver becomes red if the insects are killed by heterorhabditids and brown or tan if killed by steinernematids (Kaya and Gaugler 1993). The color of the host body is indicative of the pigments produced by the monoculture of mutualistic bacteria growing in the hosts. Beneficial nematodes are effective against grubs and the larval or grub stage of Japanese Beetles, Northern Masked Chafer, European Chafer, Rose Chafer, Fly larvae, Oriental Beetles, June Beetles, Flea beetles, Bill-bugs, Cut-worms, Army worms, Black Vine Weevils, Strawberry Root Weevils, Fungus Gnats, Sciarid larvae, Sod Web-worms, Girdler, Citrus Weevils, Maggots and other Dip-tera, Mole Crickets, Iris Borer, Root Maggot, Cabbage Root Maggot and Carrot Weevils (Grewal *et al.*, 2005). Steinernema and Heterorhabditis are commercially available in the U.S. With Steinernema been most widely studied because it is easy to produce. Heterorhabditis is more difficult to produce but can be more effective against certain insects, such as the white grubs, and Japanese beetles.

2.3 Protozoa

Protozoa are single-celled organisms that live in both water and soil. While most protozoa feed on bacteria and decaying organic matter, many species are insect parasites. In particular, one species of protozoa, *Nosema locustae*, is used to control grasshopper, locust and crickets on rangeland.

3. ADVANTAGES AND CONSTRAINS

Entomopathogenic biopesticides are advantageous because they have been reported to be environmentally friendly, no permissible toxic residue left on food and high host specificity which also helps in the preservation of natural predators when used in Integrated Pest Management systems. Furthermore, most can be applied with simple horticultural equipments including pressurized sprayers, mist blowers, and electrostatic sprayers however, the application equipment chosen on the cropping system. Despite these uses, many limitations abound which includes: scarce efficacy at low temperatures, irregular results, ease of removal by rain/ destruction (viruses) by sun rays, poor farmers acceptability and high cost of preparation compared to traditional pesticides. The appropriate use of microbial biopesticides solitarily or in IPM programmes can reduce or eliminate the frequent use of organophosphates, pyrethroids and other conventional insecticides in the management of insect pests of crops. Therefore there is need to develop and formulate strains that will be effective against specific insect pests in our own agro-ecological zones without any harm to the environment.

4. SUMMARY AND CONCLUSION

Eco-friendly entomopathogenic biocontrol agents used either solitary or incorporated into an Integrated Pest Management Programme, have been reported to significantly reduce the population densities of many economic pest in the developed World. This method of pest management produces good quality food devoid of detrimental toxic residues that could pose health problems to the consumer. Unfortunately, in most developing countries like Nigeria, farmers' knowledge of biological control is very low and as such, scientists in research Institutes and Universities, policy makers, extension specialists and Public sectors should come up with modalities to intensify the use of entomopathogenic pesticides instead of over reliance on chemical pesticides. Participatory pilot projects be established where some of these pesticides formulated from local materials and applied using simple equipment can be demonstrated to enhance farmer's awareness and acceptability.

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Plate 1. Effect of *B. bassiana* *S. litura* larva.

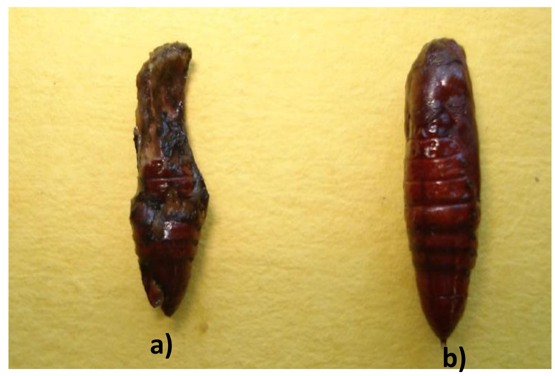

Plate 2. Activity of *B. bassiana* on *S. litura* pupa

[a) deformed and b) healthy (Malarvannan, *et al*., 2010].

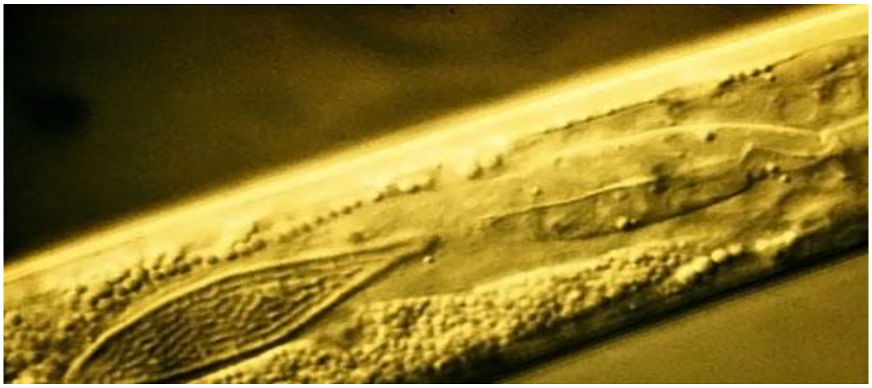

Plate3. Symbiotic bacteria observed in intestinal tract of *Steinernema carpocapsae.* (Bedding, 1982).

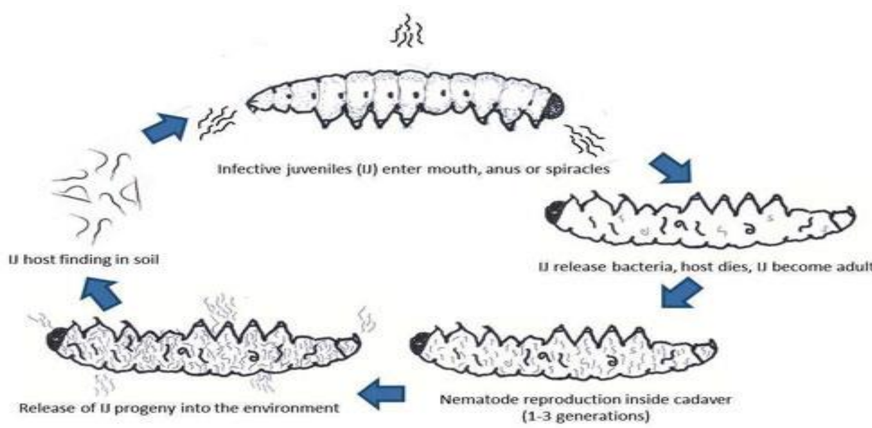

Figure I. Generalized life cycle of entomopathogenic nematodes (Tofangsazi *et al.*, 2012).

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